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ARGONNE NATIONAL LABORATORY
P. O. Box 5207
Chicago 80, Illinois

BIOLOGICAL AND MEDICAL DIVISIONS
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Editor
Austin M. Brues, Director, Biological Division

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Administration

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

Dr. Howard H. Vogel, Jr., has joined the staff as an associate biologist. He received his Ph.D. degree in zoology from Harvard University in 1940, and served as chairman of the biological science staff of the College of the University of Chicago for the past two years. Dr. Vogel is currently investigating the effects of X-irradiation on the fighting behavior of male mice, and will work with Dr. Brues and Dr. Zirkle on the neutron toxicity program.

Mr. George A. Sacher, Jr., has rejoined the staff as an associate scientist in the Special Problems Group. Mr. Sacher was a consultant with the Laboratory and an AEC fellow in the Department of Physiology, University of Chicago, from 1948 to 1950. His research will be concerned with experimental and theoretical investigations of the effect of radiations and other agents, e.g., nitrogen mustard, on the growth and survival of animals.

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Editorial

Mrs. Antreen Pfau
Miss Helen Supert

The following paper was prepared for publication and will be included in the National Nuclear Energy Series (mpts), Division IV, Volume 22C:

Sacher, G. A. The survival of mice under duration-of-life exposure to X rays at various dose rates. CH-3900 (unclassified).

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TALKS

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Schubert, J.: The Biological and Medical Aspects of Atomic Energy. Meeting of the Atomic Energy Institute, Museum of Science and Industry, Chicago, Illinois, February 4, 1950.

Allen, J. G.: The Dynamics of Coagulation. Society of University Surgeons, Philadelphia, Pennsylvania, February 10 to February 12, 1950.

Buchanan, D. L.: The Use of Isotopic Tracer Materials in Biology. Southern Wisconsin Education Association, Madison, Wisconsin, February 10, 1950.

Smith, D. E.: Biological Applications of Atomic Energy, Meeting of the Atomic Energy Institute, Museum of Science and Industry, Chicago, Illinois, February 18, 1950.

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Allen, J. G.: Tests of Liver Function. Kansas City Academy, Kansas City, Missouri, March 17, 1950.

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Biometrics

Mr. Sylvanus A. Tyler

PROGRESS REPORT: DETERMINATION OF THE LD₅₀ BY USE OF
PROBIT, ANGULAR, AND LOGIT TRANSFORMATIONS

by

S. A. Tyler and Joan Gurian

To aid in determining the dependence of response upon dosage and associated statistics for assays that admit quantal responses, appropriate statistical techniques were collected from previously published reports⁽¹⁻³⁾ and expanded into a more complete computational guide.

A short discussion of the probit, angular, and logit transformations is given and an example typical of those encountered in bioassay is used to illustrate the computational procedure associated with the use of each transformation in determining the characterizing parameters of the dosage versus response relationship. The LD₅₀ and corresponding standard error are computed and a method useful in comparing several probit regression lines is discussed.

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PROGRESS REPORT: A COMPARATIVE STUDY OF THE TIME PATTERNS OF Sr^{89} EXCRETION CHARACTERIZING VARIOUS LABORATORY ANIMALS

by
S. A. Tyler and Joan Gurian

Many studies have been made to determine the time patterns of injected radioisotopes in laboratory animals. In an attempt to determine the excretion functions characterizing different species and to compare species parameters so obtained, the excretion data from many completed experiments are being collected and analyzed.

This work has been started for the dog and goat studies in which Sr^{89} was the injected isotope. The excretion data used were taken from the experimental findings of M. N. Swift and C. L. Prosser.^(1,2) The method of exponential fitting,⁽³⁾ i.e., of fitting by sums of exponential terms, is employed to describe the excretion-time behavior and to provide a method by which an estimate of the total excreted material can be obtained. For purposes of comparison, the power function fit and other related methods of fitting are applied to the data. Comparison of the sums of squared deviations of the calculated values from the experimental points for the excretion models used indicate that the closest approximation to the empirical data is achieved by the exponential fitting method.

The constants of the fitted excretion equations for comparable fitting models are remarkably similar in the dog and goat.

The symbols used in Tables 1 and 2 are defined as follows:

- E_t = Excretion on day t in per cent of injected dose
- E_t^C = Expected excretion on day t from power function derived by least squares
- E_t^x = Expected excretion on day t from modified power function*
- E_t^d = Expected excretion on day t from adjusted power fit**
- E_t^e = Expected excretion on day t from exponential fit
- Q_C = Expected total excretion (in per cent) from power function derived by least squares
- Q_x = Expected total excretion (in per cent) from modified power function

*Modification of the least square log-log fit was derived by G. Sacher.⁽⁴⁾

**Method of successive approximations by Taylor's expansion.⁽⁵⁾

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Q_d = Expected total excretion (in per cent) from adjusted power fit

Q_e = Expected total excretion (in per cent) from exponential fit

Figures 1 and 2 are illustrative of the data given in Tables 1 and 2.

Table 1

DAILY EXCRETION OF SR^{89} IN PER CENT OF THE INJECTED DOSE FOR GOATS
GROUP 1 - 7 GOATS
(Maximum daily excretion on first day)

Time in days (t)	Original data (E_t)	Log-log fit (E_t^C)	Modified power fit (E_t^X)	Adjusted power function (E_t^d)	Exponential fit (E_t^e)
1	21.3814	36.1192	38.2773	35.0108	21.5973
2	12.5286	9.2628	9.8162	9.4802	12.4026
3	7.4957	4.9168	5.2105	5.1610	7.4793
4	4.6271	3.2418	3.4355	3.4599	4.7697
5	3.1780	2.3754	2.5173	2.5669	3.2207
6	1.7464	1.8533	1.9640	2.0226	2.2919
7	1.3560	1.5048	1.5948	1.6561	1.7033
8	0.9918	1.2609	1.3362	1.3975	1.3080
9	0.8999	1.0809	1.1455	1.2053	1.0283
10	0.7252	0.9398	0.9960	1.0530	0.8212
$\sum_{t=2}^{10}$	33.5487	26.4367	28.0168	27.9660	35.0250
$\sum_{t=1}^{10}$	54.9301	62.5557	66.2941	62.9770	56.6223

SUMS OF SQUARED DEVIATIONS

$$\sum_{t=1}^{10} (E_t^C - E_t)^2 = 237.27$$

$$\sum_{t=1}^{10} (E_t^d - E_t)^2 = 202.77$$

$$\sum_{t=1}^{10} (E_t^X - E_t)^2 = 300.26$$

$$\sum_{t=1}^{10} (E_t^e - E_t)^2 = 0.63$$

EQUATIONS OF FIT

$$\begin{aligned} E^C(t) &= 15.304 t^{-1.239} \\ E^X(t) &= 16.218 t^{-1.239} \\ E^d(t) &= 15.352 t^{-1.1895} \\ E^e(t) &= 5.6 e^{-0.1967 t} + 33.5 e^{-0.6785 t} \end{aligned}$$

TOTAL EXPECTED EXCRETION

$$\begin{aligned} Q_c &= 85.4149 \\ Q_x &= 89.2391 \\ Q_d &= 102.3946 \\ Q_e &= 60.2657 \end{aligned}$$

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Table 2

DAILY EXCRETION OF SR⁸⁹ IN PER CENT OF THE INJECTED DOSE FOR DOGS
GROUP 1 - 6 DOGS
(Maximum daily excretion on first day)

Time in days (t)	Original data (E_t)	Log-log fit* (E_t^c)	Modified power fit (E_t^x)	Exponential fit (E_t^e)
1	20.7842	37.6273	41.3710	20.3480
2	10.9437	8.2124	9.0298	11.5382
3	5.6128	4.0441	4.4466	6.8498
4	4.2927	2.5380	2.7606	4.2184
5	2.7528	1.7924	1.9708	2.7070
6	1.3203	1.3579	1.4931	1.8019
7	1.3178	1.0758	1.1828	1.2365
8	0.6608	0.8827	0.9705	0.8688
9	0.4387	0.7440	0.8169	0.6214
10	0.4548	0.6354	0.6986	0.3970
$\sum_{t=2}^{10}$	27.7944	21.2827	23.3697	30.2390
$\sum_{t=1}^{10}$	48.5786	58.9100	64.7407	50.5870

SUMS OF SQUARED DEVIATIONS

$$\sum_{t=1}^{10} (E_t^c - E_t)^2 = 297.85 ; \quad \sum_{t=1}^{10} (E_t^e - E_t)^2 = 2.40$$

$$\sum_{t=1}^{10} (E_t^x - E_t)^2 - \sum_{t=1}^{10} (E_t^d - E_t)^2 = 432.47$$

EQUATIONS OF FIT

$$\begin{aligned} E^c(t) &= 14.4003 t^{-1.3858} \\ E^x(t) &= E^d(t) = 15.834 t^{-1.3858} \\ E^e(t) &= 8.2 e^{-0.2958 t} + 29.0 e^{-0.7107 t} \end{aligned}$$

TOTAL EXPECTED EXCRETION

$$\begin{aligned} Q_c &= 58.1100 \\ Q_x &= Q_d = 61.8262 \\ Q_e &= 51.8317 \end{aligned}$$

*The adjusted power function and the log-log fit were the same to the fourth decimal place.

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Figure 1. Average daily excretion of strontium⁸⁹ in per cent of injected dose for goats (Group 1-7 goats).

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Figure 2. Average daily excretion of strontium⁸⁹ in per cent of injected dose for dogs (Group 1-6 dogs).

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Hematology

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

PROGRESS REPORT: SPLEEN-SHIELDING EXPERIMENTS

by

L. O. Jacobson, E. L. Simmons, Edna K. Marks,
Melba J. Robson, Evelyn O. Gaston, and W. F. Bethard

In previous reports we have shown that lead-shielding the spleen^(1,2) of mice increases survival, reduces the severity of hematological effects, and hastens the recovery of the hematopoietic tissue of mice exposed to total-body dosages of X radiation of 600 r or greater. Lead protection of the spleen or appendix of rabbits exposed to 800 r or 1000 r total-body X radiation also affords a more rapid recovery of hematopoietic tissue.⁽³⁾

The effect of lead protection of the spleen on the hematopoietic recovery of rats (Sprague-Dawley) exposed to total-body doses of 600 r, 800 r, and 1000 r is followed by a more rapid regeneration of the irradiated hematopoietic tissue than in rats exposed to these doses without spleen protection. After doses of 600 r, 800 r, or 1000 r active nodules are found in the hilar and mesenteric lymph nodes of rats on the third or fourth day after irradiation in the spleen-protected animals. Active nodules in the lymph nodes are not found in rats exposed to 600 r without spleen protection before 12 or 14 days. In fact, reconstitution of the germinal centers in the lymph nodes of these lead-protected rats is qualitatively* complete before reconstitution of the bone marrow in these animals is apparent. As was true in mice⁽³⁾ the bone marrow of rats which have had spleen protection during irradiation recovers prior to recovery of the marrow of rats without spleen protection during irradiation.

EFFECT OF SPLEEN PROTECTION ON HEMATOPOIETIC
RECOVERY OF GUINEA PIGS

Guinea pigs have been given single total-body exposures to dosages of 180 r, 300 r, 310 r, and 350 r with protection of the surgically mobilized spleen. Histologic recovery of the hematopoietic system of these guinea

*This term is used because the normal histologic appearance of a lymph node does not necessarily mean that a node or group of lymph nodes has attained a normal overall size.

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pigs is being compared with the recovery of guinea pigs exposed to these dosages without lead protection of the spleen. A detailed description of these studies will be reported later. Preliminary studies indicate that recovery of hematopoietic tissue, as in mice, rats, and rabbits occurs earlier in spleen-protected animals than in those which do not have this protection.

STUDIES ON TOTAL-BODY X IRRADIATION AND PARTIAL SHIELDING OF THE SPLEEN

Seven groups of mice were given 1025 r total-body X irradiation with the exception of the spleen. The surgically mobilized spleens in each group were given 50 r, 100 r, 200 r, 300 r, 400 r, 500 r, or 668 r. Mice from these seven groups were serially sacrificed and tissues prepared for histologic study.

Only the animals that received 200 r to the spleen and 1025 r to the remainder of the body have been studied microscopically. In a few of these animals it has been definitely established through the eighth day that hematopoietic regeneration in the marrow and lymph nodes is greater than in the animals at corresponding intervals that were given 1025 r to the entire body including the spleen.

It may be stated in this preliminary report that it requires more than 200 r to suppress the capacity of the spleen to stimulate a more rapid regeneration of hematopoiesis than occurs if the total body including the spleen is irradiated.

THE EFFECT OF SPLEEN PROTECTION ON SURVIVAL OF IRRADIATED RATS

Lead protection of the spleen of rats does not afford as striking an effect on survival as this procedure does in mice. The result of such protection on survival is indicated in Table 3.

EFFECT OF AGE ON SURVIVAL OF IRRADIATED MICE WITH SPLEEN PROTECTION

The mice used in our studies^(1,2) of the effect of spleen protection on survival were between 10 and 12 weeks of age. Lead protection of the spleen of this strain was found to increase the LD₅₀ from about 550 r to 1025 r. Lorenz *et al.*⁽⁴⁾ found that the survival of C-57 black and strain A 4-week mice, irradiated with spleen protection, was essentially no different from the survival of mice irradiated without spleen protection with the same dose. However, irradiation of these strains at age 7 weeks showed that spleen protection increased survival. We have tested this phenomenon in CF-1 female

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5- to 6-week mice and found that 21 mice have thus far survived an 18-day period of observation out of 76 mice irradiated (1025 r) with spleen protection. None survived this dose without spleen protection.

These preliminary data indicate that the per cent survival of 5- to 6-week mice with spleen protection may be less than the survival of 10- to 12-week mice with spleen protection. In view of Lorenz's observation we are currently studying groups of mice (CF-1 female) beginning with age 4 weeks in order to determine if any significant difference exists between survival of the 10-week spleen-shielded animals and younger mice with spleen shielding.

Table 3

EFFECT OF SPLEEN PROTECTION UPON SURVIVAL OF
X-IRRADIATED RATS

Treatment	Number exposed	Number of survivors	Per cent survivors
700 r with spleen protection	17	14	81.8
700 r without spleen protection	12	7	58.4
800 r with spleen protection	46	25	54.0
800 r without spleen protection	42	6	14.0
900 r with spleen protection	11	0	0
900 r without spleen protection	9	0	0
1000 r with spleen protection	22	1	4.5
1000 r without spleen protection	19	0	0

REPEATED ACUTE IRRADIATION WITH SPLEEN PROTECTION

A pilot group of 37 spleen-shielded mice has now received four repeated doses of 1025 r at monthly intervals. Two of the mice still survive. Sacher(5) as well as Lorenz(6) has previously reported that mice receiving small daily doses are able to tolerate total accumulated dosages well within this range. However, a single acute dose of 1025 r is invariably fatal to CF-1 mice which do not have spleen protection, but these mice with lead protection of the spleen may tolerate four such acute exposures.

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Another pilot group of 10 mice has been treated in similar fashion, except that a smaller monthly dose of 600 r was administered. Six of the 10 mice still survive after four exposures of 600 r each.

STUDIES TO DETERMINE THE NATURE OR FACTOR PRODUCING HASTENING OF HEMATOPOIETIC REGENERATION IN ANIMALS IRRADIATED WITH SPLEEN OR APPENDIX PROTECTION

Spleen extracts: In general, it appears that this phenomenon of hastened regeneration observed in mice with lead protection of the spleen has either a humoral or cellular basis. Studies on the effect of injection of lyophilized saline extracts of fresh beef spleen on mice that received 1025 r X irradiation with and without spleen shielding have been performed. Mice were injected at 3-hour intervals for 4 days with an extract containing the equivalent of 0.2 g of beef spleen, or approximately twice the weight of a normal mouse spleen, in each injection. Control animals were given sham fluid injections of isotonic saline. Animals were then sacrificed and autopsied at daily intervals, and tissues are now being prepared histologically. A report of these findings will be made at a later date.

Using a different extraction technique a lipid fraction of the spleen has also been prepared and a comparable experiment has been set up to test the effect of this extract on the X-irradiated mouse.

Effect of spleen implant on hematopoietic recovery: To test the regenerative action of spleens from very young mice on the hematopoietic tissue of the X-irradiated mouse, mice weighing 20 g were given 1025 r total-body X irradiation without spleen shielding. Immediately following X irradiation spleens from 4 young mice (4 to 5 days old) were inserted into the peritoneal cavity of each of 12 X-irradiated animals. These animals were sacrificed on the 7th and 8th day after irradiation and the tissues were prepared for microscopic study. Examination of those tissues available indicates that hematopoietic regeneration in these animals was more rapid than in animals that were exposed to total-body X radiation without spleen protection and without intraperitoneal insertion of normal spleen from very young mice.

These preliminary findings suggest that the factor or factors influencing a more rapid regeneration of hematopoietic tissue from irradiation in the spleen-protected animals is humoral rather than cellular.

THE EFFECT OF SPLEEN PROTECTION ON NITROGEN MUSTARD TOXICITY

Nitrogen mustard exerts its major effect on blood-forming tissue within a few minutes after intravenous administration. With this in mind an experiment was designed similar to those of the X-ray, spleen-shielding series. In preliminary experiments it became apparent that the splenic pedicle

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of mice could be clamped off for as long as 20 minutes without the occurrence of appreciable necrosis of the splenic tissue. After a 10-minute clamping period, no significant cytological changes were discernible microscopically. It was also apparent from preliminary studies that if the spleen was clamped for a period of 10 minutes and nitrogen mustard given immediately after the clamp was placed on the pedicle, the destructive changes in the spleen that regularly follow intravenous HN_2 administration to the intact mouse were minimized. CF-1 female mice weighing approximately 20 g were divided into four groups. Group 1 consisted of normal controls, group 2 animals received a single intravenous (tail vein) injection of nitrogen mustard (methyl bis (β -chloroethyl amine) hydrochloride) in a dose of 0.01 mg per g. Group 3 mice were anesthetized with Nembutal, the spleen exteriorized, and the pedicle clamped for 10 minutes. After release of the clamp the spleen was returned to the abdomen and the abdominal incision sutured. Group 4 mice were anesthetized and the spleen exteriorized. The pedicle of the spleen was clamped and while the blood flow to the spleen was thus shut off, a dose of 0.01 mg per g of nitrogen mustard was given intravenously into the tail vein. The pedicle clamp was removed after 10 minutes, the spleen was returned to the abdomen, and the incision sutured. Mice from the four groups were sacrificed at intervals after these procedures and representative tissues were prepared for histologic study. Preliminary histologic findings indicate that the hematopoietic tissues of group 2 mice (lymph node, spleen, thymus, and bone marrow) which received 0.01 mg per g of nitrogen mustard without clamping of the splenic pedicle were destroyed and were still atrophic without evidence of regeneration at 5 days. None of these mice survived beyond this interval. In group 4 animals which received the same dose of nitrogen mustard but had the spleen pedicle clamped for 10 minutes (at the time of mustard injection and for 10 minutes thereafter) regeneration of hematopoietic tissue in the lymph nodes, bone marrow, and thymus was well under way at the fourth day interval. No animals from this group survived beyond the fourth day. These preliminary findings indicate that the destructive changes produced in the hematopoietic system by nitrogen mustard can be stimulated to more rapid recovery by a technique similar to that employed in the X-ray, spleen-shielding experiments.

EFFECT OF REMOVAL OF THE LEAD-PROTECTED SPLEEN AT INTERVALS AFTER IRRADIATION

The findings^(1,2) that lead protection of the spleen during exposure to total-body X radiation so markedly reduced mortality in young adult CF-1 mice suggested an experiment to determine the length of time the lead-protected spleen must be left intact to insure survival of the irradiated animal.

Mice were divided into four groups consisting of controls (group 1), operated controls (group 2), mice exposed to 1025 r without lead shielding of the spleen (group 3), and mice with lead protection of surgically exteriorized

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spleen during exposure to 1025 r (group 4). At chosen intervals half of each group was splenectomized and the animals were observed to determine the effect on survival. A number of such experiments has been performed, and splenectomies were done at the following intervals relative to irradiation:

Splenectomy immediately prior to irradiation

Splenectomy immediately following irradiation*

Splenectomy 12 hours after irradiation*

Splenectomy 1 day after irradiation

Splenectomy 2 days after irradiation

Splenectomy 3 days after irradiation

Splenectomy 4 days after irradiation*

Splenectomy 5 days after irradiation*

Splenectomy 6 days after irradiation*

The results of those experiments which are complete indicate that no mice in group 3 (no original lead protection of spleen) survive whether or not splenectomy was performed at any interval. On the other hand survival of group 4 animals (lead protection of spleen during irradiation) occurs even if the spleen was removed 1, 2, or 3 days after irradiation. These preliminary findings indicate that the critical period during which the lead-protected spleen exerts its effect is probably within the first 72 hours after irradiation. This suggests that the factor responsible is not "seeding" since histologic study revealed that reconstitution of hematopoietic tissue at this interval was negligible. These observations suggest that the mechanism is a humoral factor coming from the lead-protected spleen or a removal of depressive or toxic substance by the lead-protected spleen. Completion of the scheduled splenectomy experiments may indicate that the period during which the spleen must remain intact to insure survival of the animal may be even less than one day.

STUDIES ON LEAD PROTECTION OF OTHER PARTS OF THE BODY

To test whether the hastened recovery of hematopoietic tissues following shielding of the spleen in mice and rats and the spleen and appendix in the rabbit is a specific capacity of the spleen and appendix or whether other protected tissues also afford some degree of enhanced recovery, other organs and regions of the body have now been shielded and the animals sacrificed and autopsied for histologic study. Only a few tissues are available

*Experiments in progress.

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at this time. Study of the hematopoietic tissues of mice which had lead protection of the surgically exteriorized kidney during total-body exposure of 1025 r X radiation indicates that no recovery has occurred by 5 days. Mice which had spleen protection while exposed to this dose, as reported earlier(3), had definite indications of recovery in both the bone marrow and lymph nodes by this interval after irradiation. Preliminary histologic studies on a few mice which had lead protection of part of the surgically mobilized liver or lead protection of the intestine including the lymph nodes at the root of the mesentery indicate that lead protection of these tissues does hasten hematopoietic regeneration but possibly not to the degree that spleen protection does.

STUDIES ON THE EFFECT OF STREPTOMYCIN ON HEMATOPOIETIC REGENERATION IN X-IRRADIATED ANIMALS

Animals were exposed to 1025 r using the standard irradiation techniques discussed in previous reports.(1,2) One group had lead protection of the spleen during irradiation, another group had no spleen protection, and a third group was splenectomized immediately before X irradiation. Beginning immediately after exposure dehydrostreptomycin in a dose of 6000 gamma was initiated subcutaneously and continued through 9 days or until death of the animal. The animals were sacrificed at 5-, 6-, 8-, and 9-day intervals and prepared for histologic study by the usual technique.

Only a few animals from these groups have been examined through the 9th day, and no effect on hematopoietic regeneration was noted in either group. The hematopoietic recovery in animals with lead protection was essentially complete as reported previously, whereas in animals without spleen protection but with streptomycin no hematopoietic recovery was apparent.

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MODIFICATION OF LEAD SHIELDS FOR PROTECTION OF THE SPLEEN AND APPENDIX DURING TOTAL-BODY X IRRADIATION

by
Edna K. Marks

ABSTRACT

In a previous report⁽¹⁾ a description was given of lead shields used to protect the spleen or appendix of rabbits during total-body X irradiation. Since that time, the shields have been modified.* The changes that were made contribute to better protection of the shielded organs and less trauma to the tissues.

The spleen shield (Figure 3A and 3B) consists of two parts that fit together by means of overlapping flanges. This arrangement prevents compression of the splenic pedicle and the entrance of X rays. The wall of the shield is 1/4-inch thick.

The appendix shield (Figure 3C and 3D) is similar to that for the spleen. The larger opening at one end permits the passage of the proximal end of the appendix and the narrow slit, with overlapping flanges, allows the mesentery and blood supply of the appendix to remain intact and prevents the appendix from slipping out of the shield during irradiation.

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*Redesigned by Mr. Albert Pearl, Central Shops, Argonne National Laboratory.

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A

Spleen Shield

B

Spleen Shield

C

Appendix Shield

D

Appendix Shield

Figure 3. Lead shields for protection of rabbit spleen and appendix.

PHOTOGRAPHED BY JANE K. GLASER

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PRELIMINARY REPORT: THE EFFECT OF SPLEEN AND APPENDIX
PROTECTION ON THE SHWARTZMAN REACTION

by

M. O. Goldman, L. O. Jacobson, and Melba J. Robson

When a preparation of meningococcus endotoxin is injected intradermally into a rabbit and 24 hours later is injected intravenously, there develops a severe hemorrhagic necrotic reaction at the intradermal site within two to six hours. This reaction was first described by Shwartzman in 1927 and was called a phenomenon of local tissue reactivity.⁽¹⁾ The reaction can be elicited by many unrelated bacterial endotoxins as well as by certain nonbacterial substances⁽²⁾ and is thought to be a nonspecific reaction not involving known immunological mechanisms. Recently Becker, by the use of total-body X irradiation, nitrogen mustards, or benzol, was able to obtain suppression of this reaction if these agents were administered in large doses before the intradermal injection.⁽³⁾ From this he postulated that the mechanism of suppression by these agents is exerted through their common suppressive action on the reticulo-endothelial system, primarily the vascular endothelium, and he suggested that the endothelial cells are rendered anergic and not able to react to the active principle in a self-destructive manner. The purpose of the present work is to investigate possibilities of altering this suppression with the view of finding possible alternate explanations for its mechanism. To this end total-body X irradiation and meningococcus endotoxin were used according to the methods of Becker.

Three sets of experiments were performed. The first experiment was designed to determine whether irradiation exerts its suppressive action through local effects on the skin. Rabbits were given local X irradiation (800 r) to the skin, and the Shwartzman reaction was carried out at this site five days after irradiation. It was found that local tissue irradiation failed to suppress the reaction.

In the second set of experiments the role of lymphatic tissue was investigated, and it was decided to shield some large lymphatic deposit in the body during total-body X irradiation (800 r) to determine whether such preservation would result in a failure of Shwartzman reaction suppression. Rabbits were placed in several groups, including groups which were operated at the time of irradiation, and a lead or a sham shield was placed around either the spleen or appendix of the animal during the entire period of exposure. This technique is identical to that employed in the experiments by Jacobson *et al.*⁽⁴⁾ in experiments on the role of the spleen and appendix in antibody formation. The results obtained are summarized in Table 4.

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Table 4.

THE EFFECT OF SPLEEN AND APPENDIX PROTECTION ON THE
SHWARTZMAN REACTION

X-ray dose (r)	Type of protection or manipulation	Shwartzman reaction No. of animals	
		Positive	Negative
0	None	11	
0	Appendix mobilized	9	
0	Spleen mobilized	3	
800	None		19
800	None	3	
800	Appendix mobilized		5
800	Appendix mobilized	6	
800	Appendix protected by lead shielding		1
800	Appendix protected by lead shielding	9	
800	Spleen mobilized		6
800	Spleen mobilized	2	
800	Spleen protected by lead shielding		3
800	Spleen protected by lead shielding	4	
800	Intestine manipulated		2
800	Intestine manipulated	4	
800	Thigh traumatized		2
800	Thigh protected		2
800	Thigh protected	1	
800	Local X radiation to thigh	3	
600	None		2
600	Spleen mobilized		2
600	Spleen mobilized	1	
600	Spleen protected by lead shielding		1
600	Spleen protected by lead shielding	2	
1000	None		5
1000	None	2	
1000	Appendix mobilized		3
1000	Appendix mobilized	1	
1000	Appendix protected by lead shielding	4	
1000	Spleen mobilized	3	
1000	Spleen protected by lead shielding		1
1000	Spleen protected by lead shielding	3	

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The data failed to shed much light on the role of the lymphatic tissues but did indicate that the operation alone (with sham shield), which involved manipulation of the appendix or the spleen and the intestine to some extent, seemed to inhibit the Shwartzman reaction suppression (i.e., the reaction occurred). There appeared to be some tendency for the animals with lymphatic tissue protection to exhibit more pronounced reactions, but this was not statistically significant.

In the third set of experiments the role of trauma in preventing Shwartzman's reaction suppression was further investigated and in this series animals were operated upon and their intestines manipulated for five minutes before irradiation. In addition some animals were traumatized by a thigh operation. With this series it was observed that the operation and trauma to the abdominal viscera before irradiation resulted in a failure of Shwartzman reaction suppression in a high percentage of animals, while the X-irradiated controls seldom gave reactions. The thigh-operated group also failed to react.

DISCUSSION

From the results of the first set of experiments it was concluded that the phenomenon of Shwartzman reaction suppression by total-body irradiation is dependent upon some generalized change throughout the body, not upon some local alteration at the site of intradermal injection. Thus it would appear that local effects of irradiation on the vascular endothelium could not account for the suppression. What this more generalized change is dependent upon is not clear. One possibility is that the suppressive action of the irradiation is through its destructive effects on the lymphatic tissues of the body. Attempts to investigate this possibility in the second experimental group failed however to settle this problem, although some tendency in this direction was observed. It has been shown that in rabbits total-body X irradiation, in the doses used in this experiment, markedly diminishes subsequent antibody response. If, therefore, the suppression of the Shwartzman phenomenon by irradiation is through its destructive effects on lymphatic tissue, it could be postulated that the Shwartzman phenomenon is dependent upon the presence of an antibody-antigen complex in the cells at the intradermal site causing the cells to react in a self-destructive manner.

The second conclusion that may be drawn from the above data is that trauma to the abdominal viscera just before the time of irradiation prevents full suppression of the Shwartzman reaction. The mechanism that is operating here is also not clear. One possibility, using the concept of the Shwartzman reaction as a self-destructive antibody-antigen reaction, would be that trauma has a stimulating effect on the lymphatics, which are thereby rendered more resistant to subsequent X irradiation. Thus the failure of suppression would be due to a preservation of antibody response. Another

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possible mechanism might involve adrenal cortical secretions. It has been shown that ACTH given to animals shortly before the intravenous injection will completely suppress the reaction.⁽⁵⁾ Thus it might be that trauma to the abdominal viscera acts as another stress to the body which results in a relative adrenal cortical insufficiency. In this respect it is interesting to note that both irradiation and adrenal cortical injections affect the Schwartzman reaction in the same direction. Whether, however, this is through similar or quite different mechanisms is as yet unanswered.

CONCLUSION

From the above data it is concluded 1) that the suppression of the Schwartzman reaction by total-body X irradiation is dependent upon some generalized change produced throughout the body and 2) that trauma to the abdominal viscera at the time of the irradiation acts to prevent full suppression of the Schwartzman reaction. Various possible mechanisms are discussed.

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Chronic External IrradiationDr. Egon Lorenz
Miss Harriet SuttonPROGRESS REPORT: EFFECT OF SPLEEN PROTECTION
DURING X IRRADIATION

by

E. Lorenz, L. O. Jacobson, Delta Uphoff, Harriet Sutton,
and R. SchweisthalSURVIVAL OF C₃Hb MICE FOLLOWING ACUTE TOTAL-BODY IRRADIATION

The long-term survival study on C₃Hb male and female mice exposed to 900 r acute total-body X radiation with spleen protection, as previously reported,⁽¹⁾ is being continued. Four months after exposure 93 per cent of the animals have survived. After an initial weight loss they have continued to gain weight. The blood picture, which returned to normal about 2 weeks after exposure, has remained normal.

An additional experiment in which male and female C₃Hb mice are exposed to 400 r total-body X radiation, one group with spleen protection and one group without spleen protection, was started.

EFFECT OF SPLEEN PROTECTION OF MICE GIVEN TOTAL-BODY
X IRRADIATION AND CARRYING A TRANSPLANTED LYMPHOSARCOMA
IN THE SPLEEN

Four groups of male CAF₁ mice consisting of 20 to 28 animals each were used in this experiment. A lymphosarcoma was transplanted⁽²⁾ into the spleens of the mice. The first group of mice served as a control; the second was given 400 r with the spleen unprotected; the third group, 400 r with the spleen protected; and the fourth group, 900 r with the spleen protected. The tumor was transplanted 20 days before irradiation. At present, 90 days after irradiation, survival in the different groups is as follows:

Controls	68 per cent
400 r	85 per cent
400 r (spleen protected)	50 per cent
900 r (spleen protected)	65 per cent

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Gross observations on animals which died or were killed in moribund condition indicated that the lymphoma had probably generalized to the lymphoid tissue and in some cases to liver and kidneys. The white cell blood count in all groups shows very great fluctuations with a low heterophil count. At present it cannot be stated whether or not spleen protection has any influence on growth and generalization of this tumor, but histologic studies are under way. So far, indications are that the compensatory ectopic blood formation that arises in the protected spleen after total-body irradiation seems to have little effect on the spleen transplant of the lymphosarcoma.

EFFECTS OF SPLEEN PROTECTION ON THE INDUCTION OF LYMPHOID TUMORS FOLLOWING TOTAL-BODY IRRADIATION

In testing the incidence of lymphoid tumors in male C-57 black mice exposed to total-body X radiation, Kaplan⁽³⁾ found that for a given dose the most susceptible period was one month of age or younger. A total dose of 1000 r was administered in 12 consecutive equal treatments and a lymphoma incidence of approximately 60 per cent was found in the one-month age group. The same strain is being used to test the effect of spleen protection on the incidence of radiation-induced lymphoid tumors.

So far it has been found that in male mice of this strain at the age of 4 weeks spleen protection during irradiation has little effect on survival. Following a dose of 900 r with spleen protection, 100 per cent of the animals died within 8 days. Following a dose of 800 r with spleen protection at the age of 4 weeks, 100 per cent died within 13 days. According to Kaplan's Table I⁽³⁾ the radiosensitivity of C-57 black mice seems to be constant for mice at the age of one month or older. To find out whether or not mice of other strains also showed this seeming ineffectiveness of spleen protection at the age of 4 weeks, strain A mice were exposed at this age to 800 r with spleen protection. One hundred per cent of the animals died within 8 days. It seems therefore that spleen protection during total-body irradiation is ineffective in mice of that age. The reason for this seems obscure. It was noted, however, at autopsy that all mice showed edematous intestines distended with fluid. This may have caused death and may indicate a greater sensitivity of the intestinal tract of young, immature mice than that of older animals. At 7 weeks of age the C-57 black mice tolerated a dose of 700 r with spleen protection. This dose is being used in the experiments on the induction of lymphoid tumors by irradiation. One group of mice will receive a single dose of 700 r with spleen protection and a second group 700 r without spleen protection in 7 consecutive equal treatments.

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PRELIMINARY REPORT: THE VALUE OF MOUSE TESTES FOR BIOLOGICAL DOSIMETRY OF IONIZING RADIATIONS

by

A. B. Eschenbrenner, T. Wang, and Eliza Miller

INTRODUCTION

In an earlier report⁽¹⁾ the pattern of loss and recovery of testis weights following total-body exposures to acute doses of from 100 r to 400 r X radiation was presented. It was found that the minimum weight of the testes was dependent upon dose of radiation. It was also noted that the time required to effect minimum weight as well as time required for the weight to return to normal was function of the dose.

In a subsequent report⁽²⁾ it was shown that this pattern of loss and recovery of weights of the testes of mice exposed to X radiation from an 11-mev betatron was the same as that found for 186 kv radiation.

The present report is concerned with some preliminary observations on the pattern of damage and recovery of the testes of mice exposed to neutron radiation. The data from the previously reported experiments are included for comparison.

EXPERIMENTAL PROCEDURE

The X-irradiation conditions were as follows: 186 kv; 20 ma; additional filtration, 0.25 mm Cu and 0.55 mm Al; focus to middle of mouse, 50 cm; and dosage rate, 64 r per minute.

Irradiation conditions for mice exposed to radiation from the 11-mev betatron were as follows: The mice were placed in annular rings in relation to the target so that one group received 165 r (dose rate 37 r per minute) and another group received 300 r (dose rate 37 r per minute) as measured with a Victoreen chamber.

Irradiation conditions in which mice were exposed to neutrons of an average energy of 4 to 5 mev produced by the cyclotron of the Carnegie Institute of Terrestrial Magnetism* were as follows: The mice were confined in plastic cages at a distance of approximately 60 cm from the target. The maximum γ background from this cyclotron in this position of the cages is estimated to be approximately 30 per cent of the total dose. Dosages in "N"

*The assistance of Dean B. Cowie of the Carnegie Institute is gratefully acknowledged.

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were measured with a 100-r Victoreen chamber, and the mice received a total dose of 29 "N" at the rate of approximately 3 "N" per minute.

At weekly intervals following the total-body radiation, which was given when the animals were 3 to 9 months of age, 4 irradiated mice and 4 nonirradiated mice of the same age were killed. The testes were carefully dissected free of fat and epididymis and weighed on a torsion balance.

RESULTS

The course of loss and recovery of weight of the testes of the irradiated mice are shown in Figure 4. In this figure testes weights are expressed as per cent of weight of testes of nonirradiated mice of the same age. It does not seem that any significance should be attached to the deviation of the recovery curve in mice exposed to neutrons. The mice exposed to neutrons vary in age from 4 to 9 months whereas the other mice were 3 months of age at the time of exposure. Furthermore, there may have been appreciable difference in doses received by mice in the 2 cages. Although detailed histologic examination has not been completed, cursory examination has revealed no difference in histologic effects from the different radiations used. The close similarity of the curves for the different radiations strongly suggest the same biologic response. Likewise, in a group of mice exposed to X rays from a 50-mev betatron at the National Bureau of Standards, preliminary data indicate a similar damage and recovery of the testes. This study is not complete, however, and the final data will be given in the next quarterly report. It is concluded that the response of the mouse testis to irradiation may prove to be a valuable tool for subsequent studies on the relation between biologic effect and physical dose of different ionizing radiations.

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Figure 4. Weight changes in the testes of mice following total-body irradiation.

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QUANTITATIVE HISTOLOGIC ANALYSIS OF THE TESTES OF MICE FOLLOWING A SINGLE TOTAL-BODY EXPOSURE TO X RAYS

by

A. B. Eschenbrenner and Eliza Miller

ABSTRACT

The histologic findings in the testes of mice examined at weekly intervals following a single exposure to 400 r X rays were reported previously⁽¹⁾ as general impressions. Quantitative histologic analyses have now been completed on the preparations reported at that time and form the body of the present report.

Groups of LAF₁ male mice received an acute dose of 400 r total-body X radiation at 3 months of age. The irradiation conditions were as follows: voltage, 186 kv; current, 20 ma; filters (besides inherent filtration of the tube), 0.25 mm Cu and 0.55 mm Al; focus to middle of mouse, 50 cm; and dose rate, 64 r per minute.

Four irradiated mice and 4 nonirradiated mice of the same age were sacrificed at weekly intervals up to and including 12 weeks following exposure. The testes of the mice were fixed in Zenker-formol for 4 to 5 hours, washed for 12 to 20 hours in running water, and placed in 70 per cent ethyl alcohol for several days. Each was then cut into halves. These were dehydrated in ethyl alcohol and embedded in paraffin. The sections were cut 7 μ in thickness and stained with hematoxylin and eosin. Quantitative analysis of the testes was done by means of Chalkley's method⁽²⁾ as described in an earlier publication.⁽³⁾ This refers to the methodology and also to criteria used for identification of various cellular elements of the testes.

The results of the quantitative analysis of the cell population of the seminiferous tubules of the testes of the irradiated mice are shown in Figures 5 and 6. Figure 5 shows the population of a given cell stage of spermatogenesis plotted as a per cent value of the population found in normal testes. In Figure 6 the curves of cells in different stages of spermatogenesis are superimposed.

The histologic findings in these experiments are of importance with regard to considerations of both the mechanism of the biologic effects of radiations and the normal histology of the spermatogenic elements. The near absence of morphologic evidence of cell death is striking. This is true even when the testes weights are reduced to 40 per cent of normal following exposure to 400 r. When it is considered that this decrease of testes weight is due entirely to decrease of quantity of spermatogenic elements, the interstitial tissue and supporting stroma remaining normal in

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Figure 5. Cell population of a given cell stage of spermatogenesis plotted as per cent value of the population found in normal mouse testes.

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Figure 6. Results of the quantitative analysis of the cell population of the seminiferous tubules of the of irradiated mice. The curves of cells in different stages of spermatogenesis are superimposed.

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total amounts, it is even more striking than is at first apparent. A second significant histologic finding is that the depopulation of the spermatogenic elements is due to the selective sensitivity of a single cell type, the spermatogonia. Furthermore, this is due not to destruction of spermatogonia, as mentioned, but to an inhibition of mitotic activity for a period of between 2 and 3 weeks. Mitotic activity of spermatogonia in which they reproduce their own kind is considered to be similar to somatic mitosis. On the other hand, the maturation and division of progeny of the spermatogonia is considered to be peculiar to germinal cells. In the normal testis it is generally considered that during the maturation process in which spermatogonia develop into primary spermatocytes, it is a heterotypic mitosis in which there is a simple allocation of the 4 sets of chromosomes to 2 daughter secondary spermatocytes. On a morphological basis the division of secondary spermatocytes each into 2 spermatids (monads) is considered to be a homeotypic mitosis. This has been designated as homeotypic because the daughter spermatids each receives one-half of each of a pair of chromosomes from the spermatocyte, as in the case of somatic mitosis. The lack of finding of a retardation in this cell division in the present experiments suggests that the morphologic similarity between this and somatic cell divisions is not a true reflection of the physiology of this cell division because of the difference in sensitivity to ionizing radiations.

It will be noted in Figure 5 that the slopes of the curves representing decrease of each spermatogenic element are parallel. This strongly suggests that the irradiation administered had no discernible effect upon the rate of spermatogenesis of various stages from spermatogonia to spermatozoa. The finding of a difference in slopes of the curves representing repopulation of various spermatogenic elements may only be a reflection of a normal difference in the time required to proceed from one stage of spermatogenesis to the next, although this is pure speculation.

The observation that spermatogonia resting nuclei are enlarged just preceding the return of mitotic activity of spermatogonia suggests that these cell nuclei are enlarging preparatory to going into mitosis but that for some reason or another are unable to do so. It is natural to assume that a repair process is going on which involves some metabolic systems which are essential to mitotic activity, although there is no morphologic evidence to support this assumption. Similar large spermatogonia nuclei were seen in the testes of mice chronically exposed to 4.4 r to 8.8 r given in 8 hours daily for 12 to 16 months. They were also occasionally seen in nonirradiated testes of the currently reported experiments.

The earliest observation made in these experiments was 24 hours following irradiation. At this time there were no spermatogonia in mitosis. On the other hand, the progeny of spermatogonia, the spermatocytes, decreased in numbers only gradually over a period of several days following irradiation. This is interpreted to indicate that at least the majority of

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resting spermatogonia which were present at the time of irradiation were not prevented from developing into spermatocytes in a normal fashion. The numbers of cells in specific stages of spermatogenesis remained at normal values for a period of time required to develop into a successive stage before their populations diminished. This suggests that in addition to the absence of a finding of a morphologic effect upon postspermatogonial spermatogenesis one can also assume an absence of an effect of radiation on the time required for cells to pass from one stage to the next.

The length of time (between 14 and 21 days) required for the spermatogonia to recover from the mitosis-inhibiting effect of irradiations is rather striking. If it is true that the division of spermatogonia to reproduce their kind is similar to that of the division of somatic cells, a similar irradiation effect should be observable in other tissues. Experiments have been initiated to determine if this is the case.

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PROGRESS REPORT: EFFECT OF IRRADIATION ON A
TRANSPLANTABLE MOUSE LYMPHOSARCOMA

by
Joanne Hollcroft and E. Lorenz

In a previous report⁽¹⁾ a comparison was made of the effects of internally administered α radiation and externally administered X radiation on a transplanted mouse lymphosarcoma. Further studies have been devoted to a determination of whether the radiation effect is direct or indirect (systemic). Table 5 is a summary of the experimental data. Column 5 gives the average per cent tumor regression and column 6 the number of cures, i.e., the number of animals in which the tumor has not recurred to date (2 months after treatment). Experiments 1 to 9 were designed to study the effect of irradiating only the tumor or only the body with the tumor shielded with the same dose and with different doses. No marked average regression was seen following 400 r or 1300 r irradiation of the tumor or following 400 r irradiation of the body. However, when the tumor was treated with 1000 r daily for 4 days, marked regression was observed in all animals and there was one "cure" in ten. In other experiments an attempt was made to establish the optimum body and tumor dose for tumor regression. Little difference was seen when the tumor was given 2000 r, 1300 r, or 800 r as long as the body was given 100 r. An attempt was made to localize the tissue which, when irradiated, produced the systemic effect but no single organ or group of organs could be found at this time.

Experiments 13 to 15 were studies on the effect of spleen protection during total-body irradiation on tumor regression using the technique described by Jacobson, et al.⁽²⁾ Spleen protection apparently does not interfere with the tumor regression. With 400 r tumor regression was not significantly different from that produced with 400 r in mice that did not have spleen protection. With 800 r and spleen protection, the tumor did not recur in 6 out of 7 animals. From the preceding experiments it seems that the systemic effect on the tumor is due to the general toxic effects produced when tumor and total body are irradiated. Therefore, spleen protection apparently does not reduce general radiation toxicity. This conclusion is supported by the findings of Straube et al.⁽³⁾ who found that cysteine, which reduces radiation toxicity, also partially protects tumor transplants in rats that were given total-body X radiation. The last experiments (16 to 22) indicate that the systemic effects of total-body irradiation become less and less pronounced as the time interval between the treatment of the total body alone and the tumor alone is increased. Thus, when the tumor is irradiated with 400 r, 6 to 24 hours after the body has received a dose of 400 r, only slight regression was observed. The amount of regression increased when the interval was reduced to 4 hours but was less than the regression observed when tumor and body were irradiated simultaneously. The results were the

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Table 5

SUMMARY OF EXPERIMENTAL DATA

Experimental treatment	No. animals	No. surviving treatment	No. unpalpable tumors	Average: $\frac{\text{Minimum tumor size}}{\text{Tumor size at treat-}} \times 100$ ment	Cures
1. 400 r* to tumor alone	16	16	1	59 ± 31	0
2. 400 r* to body with tumor shielded	20	16	0	85 ± 27	0
3. 1300 r to tumor alone	20	20	3	25.6 ± 21	0
4. 1000 r to tumor alone for 4 days	10	10	10	0	1
5. 400 r* to body alone + 100 r to tumor	9	9	0	83 ± 17	0
6. 100 r to total body + 1900 r to tumor alone	9	9	7	3.3 ± 7.2	0
7. 100 r to total body + 700 r to tumor alone	10	10	7	$.74 \pm 1.5$	0
8. 100 r to total body + 1200 r to tumor alone	10	10	4	1.1 ± 1.1	0
9. 300 r to total body + 1000 r to tumor alone	10	10	7	$.2 \pm .3$	0
10. 400 r* to upper body including tumor	10	10	2	23 ± 21	0
11. 400 r* to middle body including tumor	10	10	0	18.7 ± 18	0
12. 400 r* to lower body including tumor	10	8	0	66 ± 9	0
13. 400 r total body	19	19	5	8.2 ± 8.0	0
14. Spleen protected 400 r total body	9	9	3	$.8 \pm .7$	1
15. Spleen protected 800 r total body	7	7	7	0	6
16. Spleen mobilized (controls)	9	9	0	100	0
17. 400 r body alone + 400 r tumor alone 24 hours later	10	10	0	74 ± 19	0
18. 400 r tumor alone + 400 r body alone 24 hours later	10	10	0	58 ± 21	0
19. 400 r body alone + 400 r tumor alone 6 hours later	10	10	0	75 ± 30	0
20. 400 r tumor alone + 400 r body alone 6 hours later	9	9	0	69 ± 7	0
21. 400 r body alone + 400 r tumor alone 4 hours later	10	10	0	42.9 ± 12.2	0
22. 400 r tumor alone + 400 r body alone 4 hours later	10	10	0	33.9 ± 7.6	0

*Two-hour exposures, all others short time exposures of a few minutes duration.

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same whether the tumor was irradiated first and the body afterwards or vice versa. From these experiments it can be concluded that the systemic effect, which is apparent following simultaneous irradiation of the total body and the tumor, lasts only for a short time and may be due to a rapidly disappearing nonspecific toxic material produced by the irradiation.

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50PROGRESS REPORT: THE 30-DAY LD₅₀ FOR HYBRID GUINEA PIGS

by

E. Lorenz, Delta Uphoff, Harriet Sutton, and R. Schweisthal

Preliminary data have been given for the determination of the X-ray 30-day LD₅₀ for hybrid guinea pigs. Blood changes in animals exposed to doses of 220 r, 260 r, and 310 r have been observed.^(1,2) These studies have been concluded with findings on a group exposed to 360 r and another to 420 r (Table 6).

From this table it is apparent that the mortality observed following a single total-body exposure to 360 r is inconsistent with the rest of the data. Furthermore, in these last two groups there is a sex difference in mortality, the females being more radiosensitive than the males. Since a hybrid stock was used and the experiments were spread over a period of one year, these observations are very likely due to chance.

A statistical analysis by use of the probit method gives the following:*

When separate lines are fitted to male and female animals we obtain:

	Males	Females
LD ₅₀	2.764 ± 0.273 (581 r)	2.547 ± 0.046 (353 r)
1) Slope	1.135 ± 1.055	2.665 ± 1.054

Neither the LD₅₀'s nor the slope differ significantly.

When a common slope is fitted for both males and females, we obtain:

	Males	Females
LD ₅₀	2.652 (449 r)	2.571 (373 r)
2) Slope	1.901	1.901

The LD₅₀'s do not differ significantly and we are consequently justified in combining data for males and females.

When a single line is fitted to the combined data for males and females, we have:

	Males and females
LD ₅₀	2.611 ± 0.062 (409 r)
3) Slope	1.902 ± 0.739

*Doses were converted to logarithm.

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Table 6

THE 30-DAY GUINEA PIG MORTALITY FOLLOWING EXPOSURE
TO VARIOUS DOSES OF X RADIATION

Dose	No. of animals	Males	Females	30-day mortality		
				Males	Females	Males and females
220 r	59	30	29	8 (27%)	9 (31%)	17 (29%)
260 r	60	30	30	12 (40%)	11 (37%)	23 (38%)
310 r	60	30	30	14 (47%)	13 (43%)	27 (45%)
360 r	59	29	30	7 (24%)	12 (40%)	19 (32%)
420 r	60	30	30	15 (50%)	20 (67%)	35 (58%)

The 2σ limits on the LD_{50} are in terms of roentgens: 307 r and 543 r.

If the data are tested for heterogeneity by the use of chi-square, we find that the departures of the observed frequencies from the fitted line yield a chi-square of 6.86, which with three degrees of freedom is not quite significant at the 0.05 level. The probability of so large a departure by pure chance is between 0.05 and 0.10. If we inflate the estimated error by a heterogeneity factor = $\sqrt{\frac{6.86}{3}}$, taking account of the fact that this is determined with only three degrees of freedom, the estimated 95 per cent error limits become 206 r and 812 r for the LD_{50} .

If the data for 360 r are omitted, we obtain:

	Males and females
LD_{50}	2.537 ± 0.037 (345 r)
4) Slope	2.662 ± 0.801

In terms of roentgens the 95 per cent error limits become 290 r and 409 r. The chi-square about the fitted line is 0.1075. With two degrees of freedom this is unusually low and might be expected to be exceeded about 94 per cent of the time. It is unlikely that the estimates obtained by omitting the data of 360 r dose could be reproduced with heterogeneous animals even within the error limits given. Therefore, the data under 3) which, given the 30-day LD_{50} for males and females of all 5 exposures, are taken as the final value:

Hybrid guinea pigs, 30-day $LD_{50} = 409$ r.

with 2σ limits of 307 r and 543 r.

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This dose is at variance with data published in the literature. Bloom⁽³⁾ gives the 30-day LD₅₀ as 175 r and Haley and Harris⁽⁴⁾ give this dose as 200 r. This discrepancy is difficult to explain. The guinea pigs in our experiments were irradiated with a double tube setup⁽¹⁾ which permits a more uniform tissue dose than obtainable with a single tube. The guinea pigs came from the stock maintained at the National Institute of Health. They were received at the age of one month and were irradiated when three months old. With the exception of the group exposed to 360 r, several animals in each of the other groups, developed respiratory diseases or salivary gland infection before they reached the age of three months (both diseases are common in guinea pigs). The diseased animals were removed from the experimental groups. Only animals which were in good health, as evidenced by their weight curves, were irradiated. The question arises whether or not the low 30-day LD₅₀ doses reported by other investigators may not have been caused by animals with chronic diseases.

In a subsequent report data will be given on the effect of irradiation on the peripheral blood.

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Physiology

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

PROGRESS REPORT: THE EFFECT OF ANOXIA UPON THE SURVIVAL
OF ADULT FROGS AND DAY-OLD RATS

by

D. E. Smith, H. M. Patt, and Ella B. Tyree

Dowdy et al.⁽¹⁾ reported that subjecting rats to anoxia during exposure to lethal dosages of X radiation greatly increases their chances for survival. If the protection were directly dependent upon the degree of anoxia, we thought that an animal capable of withstanding a more severe anoxia might show an even greater radioresistance. Since frogs tolerate an atmosphere of nitrogen for a considerable period of time, experiments employing frogs were carried out to check this point. Inasmuch as the same survival curves were obtained after the irradiation of frogs in either 100 per cent nitrogen or in room air, similar experiments were carried out on day-old rats which also withstand severe anoxia.

METHODS

The frogs employed were male Rana pipiens. They were housed in glass aquaria and stainless steel tanks provided with platforms which allowed them to be either in or out of water. They were kept in a room at 23°C and were unfed.

For 10 minutes prior to and during irradiation the frogs were contained in cellulose acetate test tubes through which either 100 per cent nitrogen or room air was circulated. They were exposed to total-body radiation in pairs, one of which was subjected to the anoxia while the other was kept in room air. A pair of nonirradiated controls was similarly handled. The radiation factors were: 250 kv; 15 ma; 0.5-mm Cu and 3-mm Bakelite filters; target distance, 25.5 cm; dose rate, 240 r per minute; and total dose, 3000 to 12,000 r. Daily checks upon survival were made. An additional experiment on frogs exposed to either a 5 per cent oxygen - 95 per cent nitrogen mixture or to room air was carried out in a manner similar to those above.

At one day of age each member of a litter of Sprague-Dawley rats was removed from its mother and placed in a cellulose acetate test tube, through which either 100 per cent nitrogen or a 20 per cent oxygen - 80 per cent

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nitrogen mixture was circulated. The rats were so treated for 90 seconds before and all during the exposure to total-body X radiation. Radiation factors were: 200 kv; 15 ma; 0.5-mm Cu and 3-mm Bakelite filters; target distance, 33.1 cm; dose rate, 80 r per minute; and total dose, 500 r. After irradiation the rats were returned to their mothers. Checks upon survival were made daily thereafter.

RESULTS

The results of the frog experiments are seen in Table 7. It is clear that under the conditions of these experiments anoxia during irradiation is without effect on the survival of frogs exposed to X radiation. The control procedures revealed that the exposures to nitrogen were without effect on survival time and that spontaneous mortality was negligible during the course of our experiments.

Of 17 one-day-old rats exposed to 100 per cent nitrogen during irradiation 16 are surviving at 20 days postirradiation; while none of 17 irradiated in a 20 per cent oxygen - 80 per cent nitrogen mixture is alive. The control deaths occurred from the 9th to the 13th days after irradiation. On the basis of these limited data it seems that anoxia protects the day-old rat against the lethal effects of X radiation.

DISCUSSION

Since under the conditions of these experiments anoxia neither increased average survival time nor allowed the survival of frogs exposed to lethal doses of radiation, it would appear that a decreased oxygen tension per se does not protect all species. The reasons for the failure of oxygen lack to protect against lethal doses of X radiation to the frog are not obvious, especially when one considers the fact that day-old rats which have a similar capacity to withstand anoxia and manifest similar behavior in respect to anaerobic glycolysis are so protected. The possibility exists that the discrepancy may be due to metabolic differences between the frog and the rat or to a difference in the importance of free radical and peroxide effects in two species vastly different in radiosensitivity.

SUMMARY

Subjecting frogs to anoxia during X irradiation does not alter their radiosensitivity as measured by survival. The chances for survival of day-old rats are greatly increased by exposure to anoxia during irradiation.

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Table 7

INFLUENCE OF ANOXIA UPON SURVIVAL OF FROGS AFTER X IRRADIATION

Treatment	No. animals	Percentage survival - weeks after X irradiation							
		1	2	3	4	5	6	7	8
3000 r - room air	20	95	90	90	85	80	40	5	5
3000 r - nitrogen	20	100	100	90	80	65	65	30	5
3000 r - room air	17	100	65	54	0				
3000 r - nitrogen	17	100	94	54	18	6	0		
6000 r - room air	20	100	95	85	20	0			
6000 r - nitrogen	20	95	90	65	5	0			
6000 r - room air	23	96	78	39	0				
6000 r - nitrogen	23	96	91	26	0				
12,000 r - room air	11	91	91	18	0				
12,000 r - nitrogen	6	100	100	17	0				
12,000 r - room air	5	100	80	40	0				
12,000 r - nitrogen	5	100	80	0					
3000 r - room air	13	100	92	0					
3000 r - 5% oxygen	13	100	92	23	0				

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Special Problems

Dr. Donald L. Buchanan
Dr. Austin M. Brues
Dr. B. Vincent Hall
Dr. David M. Morris
Dr. Janet Passonneau
Dr. S. Phyllis Stearner
Dr. Theodore N. Tahmisian
Mr. George A. Sacher
Mr. Howard S. Ducoff
Miss Katherine Hamilton
Mrs. Leola Rietz
Mrs. Agnes Naranjo Stroud

PROJECTED RADIOTOXICITY EXPERIMENT

by

A. M. Brues, R. J. Hasterlik, Miriam P. Finkel, W. P. Norris,
W. E. Kisielewski, and J. Schubert

Human toxicities of radioelements are estimated by a variety of methods. In some instances, the predicted specific activity of a critical organ under given exposure conditions is used to determine "safe" exposure levels to a radioelement, by imposing the requirement of a maximum single or integrated specific activity. The specific activity which will be attained under assumed conditions of exposure may be derived from comparable experimental data where one is dealing with an isotope of an element that is not physiologically abundant. In the case of a physiological element, a calculated tissue activity may be based on the assumption that all or a substantial part of the tissue element exchanges with the absorbed isotope, or that the highest specific activity (for example, that of blood calcium or carbonate) may be realized in a limited area, e.g., of the skeleton. The several empirical and theoretical methods of calculation may give quite different answers. Considering, for instance, I^{131} , the average animal (or human) thyroid concentration may be much exceeded in certain physiologic or dietary states; a calculated whole-organ concentration may be much exceeded in the more actively metabolizing areas of a single gland; and any calculation of uptake based on turnover of iodine by the whole body may be erroneous since the half-life of I^{131} is much less than the corresponding renewal time of the body iodine.

In the case of bone-seeking α -ray emitters, in particular, whose distribution in the skeleton may differ markedly, it is dangerous to calculate toxicity on the basis of any known factors applied to the distribution pattern, as was shown in the case of plutonium and radium.⁽¹⁾ An empirical approach is therefore necessary.

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Re-examination of the data of previous experiments in this laboratory shows that the toxicity of radium, which was at the inception of the work the best understood heavy radioelement, was inadequately studied to provide a sound basis for extrapolation of other heavy α -emitters to the human being. The comparative experiments of the Rochester Group⁽²⁾ require supplementation beyond a one-year period, after which the mortality from skeletal irradiation becomes critical.

The propriety of using 1 μ g retained radium as the minimum toxic amount in the human being has been brought into question by Evans and Hempelmann⁽³⁾ on the basis of a reinvestigation of a number of human cases, since the possibility of the highest apparent toxicities being due to contaminating Ra^{228} (mesothorium 1) appears to be a real one.

With these considerations in mind, plans have been completed for an intensive study of the chronic toxicities to small animals of radium²²⁶, and some other important heavy radioelements. This investigation will employ the same experimental and statistical methods as were used in previous experiments, including the study of polonium now in progress. In addition, an attempt is being made to gain further information in cases of human radium poisoning in this area.

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PRELIMINARY REPORT: SIMULTANEOUS DETERMINATION OF TOTAL
CARBON AND CARBON¹⁴ IN SINGLE SAMPLES

by
D. L. Buchanan

In order to allow a chemical determination of carbon and a measure of carbon¹⁴ radioactivity of a single sample containing either organic or inorganic carbon the manometric carbon analysis method of Van Slyke and Folch⁽¹⁾ has been considerably modified and the essential features are outlined below.

The combustion mixture of the above-mentioned procedure has been unaltered and the Van Slyke manometer is used for the measurement of CO₂ pressure. However, this method differs from the Van Slyke-Folch method in all other respects.

The carbon sample is combusted at 600 mm pressure with CO₂-free nitrogen in the apparatus. The evolved gases pass through a microfurnace (900°C) containing silver wool and platinum foil, then through a dry-ice cold trap, and finally through a liquid nitrogen cold trap. When combustion is complete the entire system is evacuated through the traps, and the contents of the liquid nitrogen trap are transferred to a calibrated volume which is then surrounded by a thermostat at approximately room temperature. The CO₂ pressure is read on the manometer. The sample is still available as dry CO₂ gas and may be transferred to an ionization chamber or gas counter. It may also be trapped as a carbonate in a side arm of the system or converted to elementary carbon by reduction with hot magnesium in a quartz tube.

The apparatus is diagrammed in Figure 7. The operation will not be described in detail in this report. Successful development of the method required that CO₂ and only CO₂ be quantitatively trapped in f. It was soon found that at any reasonable flow rate of the gas mixture through traps g and f considerable quantities of water were escaping g, and similar amounts of CO₂ were escaping f when standard type traps were used. Lengthening and narrowing the trap dimensions failed to remedy this defect, so it appeared that the condensing gases were forming smokes. The present type traps provide for revaporization of these smokes during passage through the upper portion of each loop since only the lower half of the traps are cold. The gases may then recondense on the glass surface of the next cooled loop segment. Several loops in each trap ensure quantitative recovery.

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Figure 7. Apparatus for carbon analysis.

A-B Mercury seal type vacuum stopcocks. Cups filled with phosphoric acid.

C-M Vacuum stopcocks. Lubricated with Apiezon Grease M.

N-P Stopcocks of Van Slyke manometer.

I Microfurnace (Sargent #49090).

II Phillips gauge (Distillation Products Incorporated #PHG-1).

III Two stage oil diffusion pump (Distillation Products Inc. #GF-20W) (Drawing abbreviated).

IV Mechanical pump (Cenco Megava #92015).

a. Leveling bulb. Tubulature covered with a Kleenex dust filter.

b. Micrometer leveling bulb support (Burrell #A40-555) moving on 7/16" rod.

c. Van Slyke manometer.

d. Capillary tubing (3 mm) with ball and socket joints and 10 ml bulb. Zero mark set with a small strip of scotch tape with manometer at zero and system pumped out.

e. Calibrated bulb, interchangeable. Volume calibrated by weight of mercury needed to fill bulb, stopcock, and capillary tubing to the zero mark.

f. Liquid nitrogen cold trap. 10 mm (OD) tubing.

g. Dry ice cold trap. First loop 20 mm (OD), remaining loops 10 mm.

h and h'. Liquid nitrogen cold traps for pump and gauge protection.

i. Digestion flask (100 ml Kjeldahl) fitted with 24/40 ♂ female joint.

j. Cup for addition of combustion fluid.

k. Porcelain combustion boat.

l. Sulfuric acid trap with break off tip.

m. Vicor combustion tube (9 mm OD) filled with platinum foil and silver wool wrapped in platinum gauze.

n. Mercury manometer.

o. Side arm trap for collecting sample as carbonate or elementary carbon.

p. To counter tube and ionization chamber manifold.

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The gases present in the system during an analysis may be divided into three groups:

1. Gases that do not condense at the temperature of liquid nitrogen.
2. Gases that quantitatively condense at the temperature of dry ice.
3. Gases that do not condense quantitatively at dry ice temperature but do condense at the temperature of liquid nitrogen.

Gases of group 1 are pumped off. Gases of group 2 are removed by trap g. Gases other than CO_2 of group 3 must be removed by some other method. Fortunately such gases are not numerous. Inorganic gases that have these properties and that might be present are: HCl , Cl_2 , SO_2 , and N_2O .

Inorganic chlorides present in the sample will appear partially as HCl (this comes off to some extent before the combustion fluid is heated). The remaining inorganic and organic chloride appears as Cl_2 . Silver wool in the furnace effectively removes both gases. Essentially zero blanks were obtained with NaCl samples.

SO_2 is apparently completely oxidized to SO_3 , but N_2O is produced in yields of 80 to 90 per cent of the theoretical when amino acids are combusted. This latter gas is quantitatively decomposed to N_2 and O_2 during passage through the Vicor tube at 900°C .

Table 8 gives some results obtained on combustion analysis of three compounds. Primary standard KH phthalate was used. The alanine and cystine were reagent grade. Included in the table are some results obtained when crystalline calcium carbonate (Calcite) was acidified with dilute phosphoric acid. It can be seen from this table that the precision of the method is roughly equivalent to other commonly used carbon methods. Each analysis requires about 20 to 25 minutes exclusive of the time required for the radioactivity assay.

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Table 8

RESULTS OF CARBON ANALYSES

Substance	Carbon		Recovery %
	Theoretical mM	Found mM	
KH phthalate	2.039	2.041	100.1
	2.043	2.041	99.9
	1.840	1.838	99.9
	1.967	1.975	100.4
	1.654	1.661	100.4
	1.875	1.872	99.8
	2.010	2.003	99.7
			Av. 100.0
Alanine	2.052	2.073	101.0
	2.406	2.421	100.6
	1.727	1.742	100.9
	2.387	2.380	99.7
	2.447	2.450	100.1
			Av. 100.5
Cystine	2.985	2.963	99.3
	2.079	2.080	100.0
	1.875	1.886	100.6
	2.436	2.440	100.2
	1.319	1.328	100.7
	1.312	1.316	100.3
			Av. 100.2
Calcite (Acidified only)	2.293	2.289	99.8
	1.914	1.908	99.7
	1.606	1.599	99.6
	1.378	1.375	99.8
	1.773	1.780	100.4
	2.008	2.009	100.0
			Av. 99.9

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PROGRESS REPORT: FURTHER STUDIES ON THE PROTECTION OF
CELLS IN VITRO FROM X-IRRADIATION DAMAGE BY
SH COMPOUNDS

by
B. V. Hall

The several published experiments demonstrating the protection of organisms or cells from X-irradiation damage^(1,2) do not permit one to assess how much of the protection, if any, is afforded by the cysteine in the fluids bathing the cells. It has been shown that mouse tumor fragments treated in vitro for 15 to 20 minutes with 0.0079 M cysteine solutions and subsequently irradiated while surrounded by and slightly covered with the cysteine solution are markedly protected from lethal effects of X irradiation. The following experiment was undertaken to evaluate the importance of the extracellular cysteine in the protection of irradiated tumor cells.

Approximately uniform mouse tumor fragments weighing 12 to 14 mg were obtained as described by Hall,⁽²⁾ and 24 to 26 pieces of tumor were placed in each of four Columbia embryological dishes. To two of the dishes 2 cc of 0.85 per cent NaCl solution saturated with cystine at 27°C and to two dishes 2 cc of 0.85 per cent NaCl containing 0.0079 M cysteine were added. Previous experiments⁽¹⁾ have shown that cystine under these conditions does not affect the radiosensitivity of the tumor. The pH of the solutions was adjusted to 6.5 to 7.5 with 3 M NaOH. After 20 minutes the cystine and cysteine solutions were removed with pipettes. The cystine-treated tumors, serving as irradiated controls, were then irradiated with 3000 r of X rays, 200 kv and filtered with 2 mm of Al. Before irradiation, the cysteine-treated tumors were thoroughly washed for 2 minutes in two separate washings of 3 cc of cysteine-free physiological salt solution. They were then irradiated under the same conditions as the cystine-treated tumors but with only a minimum of fluid clinging to the pieces of tumor. Twenty tumor fragments from each dish were implanted with trocars bilaterally into subcutaneous axillary loci of 10 young host mice. At semi-weekly intervals the host mice were weighed, and 2 or 3 diameters of the tumors were measured and recorded. Tumor volumes were calculated as described previously.⁽³⁾ The data given in Table 9 and Figure 8 briefly summarize the results. For comparison in the tabulated data are included the results of in vitro X irradiation of tumor fragments in NaCl solution without cysteine or cystine, and cysteine-treated implants which were irradiated while remaining in the cysteine solution.⁽⁴⁾

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Figure 8. Tumor volumes measured at semiweekly intervals showing the influence of pretreatment with cysteine upon the growth of tumor fragments following 3000 r X irradiation.

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Table 9

COMPARISON OF THE EFFECTS OF 3000 r X IRRADIATION UPON THE SURVIVAL AND GROWTH OF TUMOR FRAGMENTS AFTER TREATMENT WITH 0.0079 M CYSTEINE

Treatment	Number of implants	Survival		Average volume (cu mm)	Days after implantation
		Number	Per cent		
Nonirradiated controls*	98	98	100	884	11
Cystine, not washed	40	17	47.5	152	21
Cysteine, irradiated in solution*	60	52	86.7	501	21
Cysteine, washed	40	33	82.5	562	21

*Data taken from unpublished cysteine-irradiation experiments Series M and N.

The data indicate that the protection from ionizing radiation afforded the tumor cells by treatment with cysteine is not due to the presence of cysteine in the surrounding solution at the time of irradiation. It is highly probable that irradiation damage to cells is largely due to intracellular ionization. The results of this experiment support the conclusion that the protection from ionizing radiation afforded cells by cysteine is an intracellular phenomenon.

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PROGRESS REPORT: COMPARISON OF THE EFFECTS OF IRRADIATION
IN VITRO WITH TRITIUM AND X RAYS ON THE GROWTH AND
SURVIVAL OF MOUSE TUMOR IMPLANTS

by

B. V. Hall and Agnes Stroud

Heavy water, D_2O , by virtue of the large mass ratio of H^2 to H^1 has many unique physical, chemical, and biological properties, such as being a respiratory poison in high concentration. Tritium (H^3) has even a greater mass ratio to protium (H^2), resulting in more marked differences in the chemical and physical properties of H_2^3O . In addition, H^3 is a long-lived, negative β emitter. The combination of these two effects should make the biological properties of tritium, at least in the higher concentrations, much different from either protium or deuterium. These experiments were initiated to investigate the effects of exposure to tritium in vitro upon the survival and subsequent growth of mouse tumor fragments, and to compare these effects with those resulting from exposure to equivalent doses of X radiation.

METHODS

The tumor used is a rapidly growing, anaplastic mouse carcinoma⁽¹⁾ that grows readily in routine transplants with usually 100 per cent takes in the Urbana-Swiss mice employed as hosts. With aseptic precautions, 7- to 14-day-old tumors were removed from stock animals and cut into 12- to 14-mg pieces with fine scissors. Care was taken to use only uniform tumor tissue, free of necrotic areas. For each dose level about 24 selected tumor fragments were placed in small sterile Erlenmeyer flasks containing 2 cc of 0.9 per cent NaCl solution made up with varying concentrations of H_2^3O . The tritium concentrations varied from 500 mc per cc to 31.25 mc per cc. The dose levels employed are listed in Table 10. The tissues were kept in the tritium solutions for 90 minutes at $28^\circ C$. After removing the tritium solution with a pipette, the tissues were washed with two changes of 0.9 per cent NaCl for 10 minutes. Tumor fragments were implanted bilaterally by means of trocars into subcutaneous axillary loci in mice weighing from 12 to 20 g. Equivalent groups of 10 mice were used in each series.

The radiation dosage from the β rays of the tritium was calculated as roentgen equivalents (RE) by means of the "61" formula of Cohn,⁽²⁾ using 5.69 kev as the average energy of the β particles resulting from the tritium decay.⁽³⁾

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Table 10

**COMPARISON OF RESULTS OF TREATMENT OF TUMOR FRAGMENTS IN VITRO
WITH TRITIUM WATER FOR 90 MINUTES AND 200 KV X RAYS**

Tritium dose in RE	Per cent "takes"	Average vol at 2 wks (cu mm)	X-ray dose in r	Per cent "takes"	Average vol at 2 wks (cu mm)
10,850	none	1	10,000	none	1
5425	none	2	6000	none	3
4890	40	57	--	--	-
4360	45	116	4000	none	1
3870	40	14	--	--	-
3320	30	44	3500	20	195
2713	55	44	3000	70	41
2310	45	86	2500	80	11
1800	100	455	2000	100	220
1357	100	474	1500	100	493
678	100	711	1000	100	839
controls	100	885	controls	100	1142

The X radiation, given at the rate of 1028 r to 1072 r per minute was obtained from a 200-kv machine operated at 15 ma. The beam was filtered with 2 mm of aluminum. The tissues, slightly covered with normal salt solution, were irradiated in shallow Columbia watch glasses.

Host tumor-bearing mice were weighed each week and the tumors were measured with vernier calipers. Usually three diameters of the tumors were measured, excepting small tumors measuring 5 to 6 mm or less. In all cases, two tumor diameters were measured. Arbitrary, calculated volumes were obtained by cubing a single diameter for the small tumors (up to 6 mm), or by multiplying three measured diameters together. When three measured diameters were not available, the smaller measured diameter was used as the third diameter if it was 7 mm or less, if it was 8 mm, then 7 mm was used as the third diameter, and if it was 9 mm or more, then 2 mm was subtracted to obtain the arbitrary third diameter. An analysis of nearly 100 tumors with three

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measured diameters has indicated that this method of arbitrarily choosing a third diameter is in fair agreement with the diameters of the majority of the tumors examined.

RESULTS

The effects of tritium or X-ray treatment upon the growth and survival of the tumor implants are shown in Table 10 and in Figures 9 to 12. The data show that exposure to tritium for 90 minutes, especially at the higher concentrations, retarded the growth of the implants and was lethal after cumulative doses of 5425 RE or more. On the other hand, 4000 r proved to be a lethal dose of X radiation. The survival curve after X ray in Figure 9 shows a steeper slope than that following tritium irradiation. The LD₅₀ dose of X ray was about 3000 r, while the LD₅₀ dose of tritium was less definite, but seemed to be about 2500 RE. At the lower irradiation dosages, tritium appeared to be somewhat more effective in retarding the growth and in killing the tumor fragments than were similar doses of X rays. However, above the LD₅₀ dose, 3500 r or 4000 r of X radiation was much more effective in killing or retarding the growth of the tumors than was equivalent exposure to β rays.

Figures 10 and 11 are semi-logarithmic plots of the growth of the tumor implants following treatment with tritium or X rays. Each point represents the average volume of the tumors growing at the time indicated. The end of each curve marks the last week of life for the surviving animals in which tumors grew. It is readily noted that the control tumors and those tumors that received the smallest irradiation were the first to show a rapid growth increment and that they killed the host soonest. Those implants that received the two highest exposures to either tritium or X rays regressed rapidly. The growth of the tumors that received intermediate doses was distinctly retarded for the first two to three weeks, then increased rapidly before undergoing terminal retardation. The initial delay is a definite radiation effect that, in general, is proportional to the amount of radiation received. Following irradiation with doses in the range of the LD₅₀ and higher, there seemed to be a characteristic marked reduction in the rate of growth of the surviving tumors during the last two to three weeks. This terminal reduction in growth rate, coupled with the initial delay, is the probable basis for the longer survival of those animals that carried the more heavily irradiated tumors.

To illustrate the difference between exposure to tritium and to X rays upon the early growth of the tumors and upon the relationship of early growth to dose, the curves in Figure 12 were drawn from the data of Figures 10 and 11. The points in these curves represent the additional time in weeks required for the implants to grow to 1000 cu mm when the

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Figure 9. Survival of tritium-treated and X-rayed mouse tumor fragments.

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Figure 10. Growth of mouse tumors following in vitro treatment with tritium.

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Figure 11. Growth of mouse tumors following in vitro irradiation with 200 kv X rays.

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Figure 12. Additional time required for tumor implants to grow to 1000 cu mm after exposure to H^3 or X rays. Time for nonirradiated control tumors to reach 1000 cu mm used as zero time.

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time for the nonirradiated tumors to reach the same size is taken as zero time. It is evident, except for one point, that delays resulting from X irradiation when plotted against dosage form a smooth curve that must approach infinity as a limit in the region of 4000 r, for after 4000 r the tumors do not grow. The points for the tritium-treated tumors did not fall on a smooth curve, so none was drawn. But, it is apparent that, especially at the higher doses, tritium is less effective in delaying growth than X rays of equivalent dosage. The scattering of the tritium values may be due to variations in the diffusion of H_2O into the tumor fragments because of unavoidable differences in size and shape and in the cellular nature of the fragments.

DISCUSSION

In view of the difficulties of tritium analysis, the variance in reported values for the average energy of the β particles resulting from tritium decay, and the uncertainties of the rough biological assay of radiation effects used in these experiments, it is not surprising that discrepancies in the effects of theoretically equivalent radiation doses of tritium and X rays should be observed. It is perhaps more surprising that the ionization due to soft β rays and medium hard X rays should give so similar results. The variation in the effects obtained with the different tritium exposures must be largely due to the fact that β radiation from H^3 is not effective, because of its low energy, until it has diffused into a tumor fragment. The central area of a tumor particle will not be subject to the action of very soft β rays until the H_2O has reached the area. The calculation of the roentgen-equivalent doses of tritium on the basis of 90 minutes is accurate to the extent that the rate of diffusion into the tumor fragment is similar to the outward diffusion rate and that the time to achieve saturation equilibrium is short in relation to the 90-minute exposure. The rate of diffusion of H_2O into the tumor mass and into cells is possibly not greatly different from the rate at which H_2O diffuses into cells, for Lucke and Harvey⁽⁴⁾ found that the diffusion rates of D_2O and H_2O were the same for Arbacia eggs.

The indication of these experiments that the higher irradiation doses of tritium are less effective than equivalent X irradiation may be partially due to the marked difference in the dose rates. The X-ray doses were given at about 1000 r per minute, while the tritium β irradiation varied from less than 8 RE per minute to a maximum of 121 RE per minute. Lea⁽⁵⁾ and others have demonstrated that the lethal effects of irradiation given at high rates are greater than the same irradiation given at low rates. Further experiments are planned to investigate the dose-rate effect of X irradiation on tumors in vitro by giving 3000 to 4000 r of X radiation during 90 minutes.

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In conclusion, it may be stated that the effects of equivalent dosages of ionizing radiation from the β particles resulting from tritium decay calculated as roentgen equivalents and X-ray roentgens from a 200-kv machine have been shown to have similar effects on the growth and survival of mouse tumor fragments.

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PRELIMINARY REPORT: RADIATION EFFECTS FROM DIFFUSE AND POINT SOURCES

by
Janet Passonneau and Katherine Hamilton

Experiments are in progress to compare radiation effects from diffuse and point sources on skin surface.

In the first experiment female CF-1 mice were treated with soft X rays (50 kv; 40 ma; target distance, 10 cm; and no filters). The animals were anesthetized with Nembutal and irradiated through perforated lead shields. The lead shields (1/16-inch thick) were designed so that the area exposed varied inversely with the dosage given (Table 11).

Table 11

DESCRIPTION OF LEAD SHIELDS USED IN SOFT X-RAY EXPERIMENT

Number of holes	Dimensions of holes	Total area of holes	Dose
1	1 1/2 x 1/2 inch	3/4 ² inch	15,000 r
6	13/64 inch diameter	3/16 ² inch	60,000 r
10	5/64 inch diameter	3/64 ² inch	240,000 r

The experiment was run in two series. In series I the animals were irradiated on the ventral surface. The mortality was high, apparently due to damage to the abdominal organs. Consequently in series II the dorsal skin surface was irradiated. The treatment and survival data are given in Table 12.

In both series the skin reactions were similar. Most of the animals that received 15,000 r showed an erythema at the treatment sites within seven days after irradiation; this was followed by loss of hair. In the case of the dorsally-irradiated animals, loss of hair was accompanied by extensive ulceration. In group 2 regrowth of hair was complete at 9 weeks while in group 6 hair regrowth was only beginning around the scarred treatment sites. In groups 3 and 7, which received 60,000 r, blisters formed at the treatment sites at 7 days. By the second week the hair was lost in most cases, and scaly bald areas remained. No hair regrowth has been observed 15 weeks after irradiation. The occurrence of the skin reaction and epilation in groups 4 and 8 coincided with that of groups 3 and 7.

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Table 12

TREATMENT AND SURVIVAL DATA FOR SOFT X-RAY EXPERIMENTS

	Group	Treatment	Number of animals	Survival at 9 weeks (number)	Per cent
Series I: Irradiated on ventral surface	1	untreated	25	24	96.0
	2	15,000 r	25	16	64.0
	3	60,000 r	25	8	32.0
	4	240,000 r	25	23	92.0
Series II: Irradiated on dorsal surface	5	untreated	26	25	96.0
	6	15,000 r	26	25	96.0
	7	60,000 r	26	23	88.5
	8	240,000 r	26	26	100.0

Soft X rays have been assumed to have low penetration in soft tissue. However, bald spots were observed on the surface opposite the exposed skin at the 240,000 r dose level. At autopsy the animals in series I showed necrotic foci in the liver and intestines that corresponded in size and location to the treated skin area.

A second experiment is in preparation in which irradiation effects from diffuse and point sources will be compared. Sprague-Dawley rats will be exposed to β irradiation from Sr^{90} . Blotting paper soaked in a solution of Sr^{90} will provide the diffuse source. The point sources will be glass beads containing Sr^{90} . An epilation-producing dose from the diffuse source will be used, and an equivalent dose given from sources of 10, 20, and 50 beads. This experiment is being done in conjunction with Mr. Walter Kisielewski of the Radiochemistry group.

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PROGRESS REPORT: EFFECT OF X IRRADIATION ON BLOOD URIC ACID IN THE CHICK

by

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

Experiments are in progress to determine the pathogenesis of the early deaths in young chicks, which occur 24 to 48 hours after X irradiation at rates above 10 r per minute. In some of the individuals that died in the initial period, precipitated urates were present around the kidney tubules, on the visceral surfaces and mesenteries, in the pericardial sac, and in the gall bladder. Uric acid and its salts, the primary end products of protein metabolism in birds, are relatively insoluble. The presence of precipitated urates in moribund chicks suggested that the plasma urate concentration had been increased. An impairment of renal function was indicated, for the kidney showed extensive cellular damage of secretory tubule cells and necrosis of the undifferentiated tubular cells that are still present in young birds.

The amount of blood uric acid in newly-hatched and one-month chicks has been determined according to the method described by Brown.⁽¹⁾ The results obtained following exposure to 1000 r at 43 and 6 r per minute are shown in Table 13. It may be noted that after exposures at 43 r per minute the most marked increase occurred between 1 and 3 hours and just prior to death. Only a small increase occurred after exposure at the lower rate. Grossly visible precipitated urates in the kidneys were present only in animals with high blood uric acid values. Although the normal level of blood uric acid appeared to be slightly lower in the older chicks, the relative increase following X irradiation was the same as in the newly-hatched birds. Survival following X irradiation indicated that the radiosensitivity of the two age groups was similar also.

The increase in blood uric acid may result from failure of renal function, or it may represent an increased rate of nitrogen metabolism associated with a normal excretory rate. Although the toxic effects of renal failure in mammals requires several days to become lethal, there is evidence that in birds a complete anuria may lead to death within a few hours. An obstructive uremia has been produced in newly-hatched birds by ligation of the ureters. Survival time of 7 such operated animals averaged less than 12 hours and all were dead within 20 hours. At autopsy, large amounts of precipitated urates were found around the kidney tubules, on the visceral surfaces, and in the pericardial sac. Increase in blood uric acid following ligation of the ureters, shown in Table 13, was as rapid as that following irradiation at 43 r per minute.

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Table 13

BLOOD URIC ACID (MG PER CENT) FOLLOWING 1000 r X IRRADIATION

Animal	No. of animals	Treatment					
		43 r/min.		6 r/min.		No X ray	
		hours	mg %	hours	mg %	hours	mg %
Newly-hatched chicks Control	26						8.2
	8	1/2	8.3				
	15	1	10.8				
	17	3	27.1				
	17	5	33.0				
	6	Moribund	60.1				
	10			3	10.2		
	12			5	18.8		
One-month chicks Control	15						6.0
	7	5	21.7				
	1	Moribund	83.8				
	9			5	12.2		
Newly-hatched chicks (Ureters ligated)	4					3	28.5
	5					5	44.2

Renal function tests are now under way, the results of which will indicate the degree of impairment of normal excretory function.

The writers wish to acknowledge gratefully the microscopic examinations of tissues by Dr. F. Wassermann.

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79PROGRESS REPORT: NUCLEIC ACID METABOLISM
IN CHILOMONAS PARAMECIUMby
H. S. Ducoff

Various lines of evidence point to a fundamental role of nucleic acids in embryonic development and differentiation, tissue regeneration, and tumor growth. These processes are profoundly affected by radiation and by the radiomimetic nitrogen mustards. Nucleic acid and protein syntheses are inhibited by nitrogen mustards.⁽¹⁾ It is very possible that an alteration in nucleic acid metabolism is involved in the mechanism of radiation damage.

Experiments are in progress to determine the turnover of P^{32} , ribose C^{14} , and aglycone C^{14} in the nucleotides of pentose nucleic acid (PNA) of Chilomonas paramecium. The effects of nitrogen mustards on this organism are already under investigation.⁽¹⁾ Chilomonas paramecium grows well in a medium⁽²⁾ consisting of ammonium salts as the nitrogen source, acetate as the carbon source, a trace of thiamine, and inorganic ions: Cl^- , $PO_4^{=}$, $SO_4^{=}$, Ca^{++} , Mg^{++} , K^+ , Na^+ , and Fe^{+++} . Under the conditions of our experiments the phase of logarithmic growth lasts about 36 hours, at which time the population is about 2×10^5 cells per cc. The growth rate then declines, but remains positive for about 36 hours more, the maximum population being about 3.5×10^5 cells per cc.

We have not as yet determined the radiation dosage at which cells are perceptibly affected, but X-ray doses up to 1600 r and P^{32} activity as high as 5 μc per cc (in 200 cc of culture) have not altered the population growth curve.

A series of experiments has been performed to compare various PNA extraction procedures. Perchloric acid extraction, by several modifications of the method of Ogur and Rosen⁽³⁾, gave variable results. In one case a culture grown in P^{32} medium was processed by cold trichloroacetic acid (TCA) and lipid solvent extraction to the nucleic acid-phosphoprotein residue stage common to the Schmidt-Thannhauser^(4,5) and Schneider⁽⁶⁾ methods. Equal portions were then extracted with:

- 1) 10 per cent perchloric acid, 20 hr at 3°C
- 2) 1 N KOH, 24 hr at 37°
- 3) 1 N KOH, 10 min. at 100°*
- 4) 5 per cent TCA, 15 min. at 100°

*Suggested by Mr. Richard T. Sato.

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Total P^{32} recoveries by each method were in good agreement, and methods 1, 2, and 3 gave very close agreement for desoxypentose nucleic acid (DNA) activity, which was about 1/5 the value for PNA by methods 2 and 3. Inorganic phosphorus, presumably derived from phosphoprotein, appears with the nucleic acids in these extracts. This "phosphoprotein" P^{32} , determined directly by 2 and 3, and calculated by difference for 1 and 4, was about 4 times the value for PNA P^{32} . Since the P^{32} was in the medium for about 7 generation times, activity figures are probably a good index of actual concentration in this experiment. The Schmidt-Thannhauser method appears preferable for extraction of ribonucleotides prior to separation on ion exchange columns, but experiments are under way to develop a method of flocculation of the precipitated DNA without resort to TCA, which absorbs strongly in the ultraviolet, thus interfering with spectrophotometric determination of total PNA.

Because of the low PNA content of Chilomonas (ca. 10 μ g per 10^6 cells or 2 mg per liter culture), recovery and measurement of individual nucleotides from ion exchange columns has been difficult. Two methods are available to improve this situation (use of added carrier nucleotide is undesirable because of the already low specific activity): 1) concentration elution of fractions known to contain the nucleotides on small columns of resin⁽⁷⁾, or 2) obtaining greater population densities. Both are being attempted.

In order to secure reliable data on the nucleotide composition of the PNA, and to locate each nucleotide in its minimum volume of eluate, a considerable quantity of pure Chilomonas PNA is being prepared by the guanidine precipitation method.⁽⁸⁾

To increase cell population density, by extending the period of logarithmic growth and confining the cells to a small volume, the chilomonads are being grown in dialysis bags, attached to standard taper joints, and immersed in large volumes of medium in flasks. This leads to development of greatly increased population densities. If necessary, the dialysis bags can be transferred to fresh media for further growth. An additional advantage of growing cells by this method is that the cells can be washed free of substrates, etc., by dialysis. For example, nitrogen may be removed so that growth cannot occur and turnover rates of P^{32} can be measured without need for correcting for new synthesis.

ACKNOWLEDGMENT

The author is indebted to Dr. John O. Hutchens, Department of Physiology, University of Chicago, for technical advice and continued interest in the investigation.

8-112-80

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THE ACTUARIAL CALCULUS OF CHRONIC RADIATION LETHALITY

by
George A. Sacher

ABSTRACT

The logarithm of the rate of mortality (Gompertz function), is (except for a short period after birth) approximately a linearly increasing function of age for all species of mammals investigated and for many other metazoan forms. If the Gompertz function is a linear function of age, all of the commonly used actuarial functions can be evaluated explicitly.

We introduce a simplified picture of the effect of exposure to radiations on the Gompertz function. The effect of single exposure may be described as consisting primarily of a displacement upward of the Gompertz function without change in slope, while the effect of duration-of-life exposure is to increase the slope of the Gompertz function subsequent to exposure. These effects have been confirmed in investigations on populations of mice and drosophila imago.

The following consequences of the above schema may be readily verified.

1. For a given species:

a. The effect of a single dose received at age x is to decrease the after-expectation to that for an age $x + q$ where q is a (complex) function of dose and other variables,

b. The effect of continuous exposure throughout life at intensity I is to decrease after-expectation according to the relation

$$1/M_I - 1/M_0 = kI$$

where M_I and M_0 are mean survival times of treated and control groups and k is the lethality coefficient. This relation is confirmed by experimental data.

2. For a second species having the same lethality coefficient as the first, and calculated from actuarially equivalent age, where the life expectations of the second and the first are in the ratio $1/k$:

a. For equal single doses the reduction of life expectation due to a single dose to the second species will also change by a factor $1/k$, and hence the per cent reduction of life expectation will be equal for the two species.

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b. For continuous exposure, we find that to reduce the life expectation of the second species in the same proportion as the reduction caused in the first by dose rate I , we must expose the second species at the dose rate κI . Thus if two species have after-expectations from actuarially equivalent ages of 2 and 40 years, respectively, and if the expectations of the shorter lived species is reduced by 50 per cent at a dose rate of 5 r/day, then $\kappa = 0.05$ and the dose rate to reduce the expectation of the longer lived species by 50 per cent would be 0.25 r/day. This result may be obtained from the formula above by substituting appropriate values of M_0 for the two species.

The above results were deduced from assumptions which are in reasonably good accord with experimental fact, insofar as data are available. It must be emphasized that the chronic lethality coefficient can be evaluated at present only from chronic lethality data, and that it is known for only a few short-lived species.

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Radiobiology Experiment Station

Dr. Norbert J. Scully
Dr. Alfred A. Maass
Dr. Cornelius W. Pettinga
Mr. William B. Chorney

PROGRESS REPORT: RADIOBIOLOGY EXPERIMENT STATION

by

N. J. Scully, W. B. Chorney, A. A. Maass, C. W. Pettinga,
R. Watanabe, and J. W. Glattfeld

Experiments with digitalis and soy bean are in progress 1) to continue to test the design of the plant growth chamber, particularly the humidity controlling refrigeration unit, 2) to permit observation of the growth and development of plants containing higher concentrations of C^{14} than those used in the preliminary toxicity experiments, (1)* 3) to obtain C^{14} -containing seeds for C^{14} toxicity studies, 4) to observe rates at which plants assimilate and lose $C^{14}O_2$ and CO_2 in a controlled environment, and 5) to biosynthesize randomly labelled C^{14} -containing plant products that may be used as tracers.

With respect to carbon assimilation during photosynthesis, preliminary results of the current studies indicate that $C^{14}O_2$ is assimilated at a greater rate than is $C^{12}O_2$ under the prevailing experimental conditions. Differences in the degree of differential uptake have been correlated with differences in light intensity.

GROWTH AND DEVELOPMENT OF C^{14} -CONTAINING PLANTS AND BIOSYNTHESIS OF TRACERS

Opium poppy, tobacco, and alfalfa were grown in the nutriculture chamber in atmospheres enriched with C^{14} . (2) Additional plants of each species were cultured under normal greenhouse conditions. All plants received identical nutriculture treatments.

The biosynthesis studies were initiated on March 15, 1950, and completed May 4, 1950, and the results are being evaluated. Preliminary assay of the activity of opium poppy capsular tissue indicates an absolute activity of 185 μc per g. The specific activity of this material was 445 μc per g. For this and other specific activity assays, we are indebted to Dr. Donald L. Buchanan of the Special Problems Group.

*Analysis of tissue indicated an absolute activity of 46 μc per g dry weight.

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EXTRACTION, ISOLATION, AND ASSAY OF C¹⁴-CONTAINING PLANT COMPOUNDS

Tobacco plants: During the last twenty years knowledge of the organic constituents of the tobacco plant has been substantially increased and methods of isolation for certain compounds have been thoroughly studied. However, no single scheme has been published for the systematic isolation of a number of the major constituents from a single sample. This is also true for a number of other plant species that are of considerable biological interest. The utility of C¹⁴-containing plant compounds in radiobiological research has emphasized the need for systematic chemical isolation of a number of products from a single sample of a C¹⁴-labelled plant species.

Owing to the complexity of the composition of plant tissues and in view of the limited knowledge of the chemistry of a number of plant constituents, it is unlikely that a system of isolation can be proposed that would be complete or applicable to all compounds of biological interest. This attempt is thus limited to the isolation of a series of certain organic compounds from C¹⁴-labelled tobacco plants. Methods of separation are based upon differential solubility in various solvents, selective absorption, and fractional distillation. The compounds that are of particular interest are nicotine, simple sugars, protein, starch, and rutin. In preliminary experiments using nonradioactive tissues the following have been isolated in fair yield: nicotine, characterized by dipicrate and silicotungstate; simple sugars in unrefined form; and starch. The other compounds have not been characterized sufficiently to conclusively demonstrate their presence. It is expected that the results of these studies will help provide means of isolating a variety of C¹⁴-containing compounds from other plant species.

Opium poppy: In cooperation with Eli Lilly and Company, substantial progress has been made in the biosynthesis of C¹⁴-labelled alkaloids from mature opium poppy plants. Initial work was concerned with the development of a solvent extraction procedure for the isolation of morphine from nonradioactive opium poppy tissues. During this quarter approximately 7.7 mg of morphine having a specific activity of about 0.5 mc per g of C¹⁴ was obtained by our modification of a solvent extraction procedure developed by Levine and Fulton.⁽³⁾ This is based on the fact that morphine is a tertiary amine having a free phenolic group that forms water soluble salts with strong acids and bases. A mixture of the opium alkaloids is obtained by combining the residues resulting from the evaporation of the aqueous and alcoholic extracts of ground poppy capsules. This residue is taken up in water and extracted with chloroform under controlled pH, in order to obtain the first crude extract. This product is then purified by extracting its aqueous solution with benzene, first at pH 2, and then at pH 10, in order to remove impurities. The morphine is exhaustively extracted with a mixture of benzene and isopropanol at its isoelectric point, pH 8.5. Pure morphine can be obtained by recrystallizing the product obtained in the last step by using a mixture of water, ether, and ethanol.

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The efficiency of the whole process is now being checked by adding a small quantity of pure unlabelled morphine to the combined residues of all the steps and repeating the procedure for the isolation. The activity of the product obtained by this method permits calculation of the over-all efficiency of the process as well as the recovery of small amounts of labelled morphine that may be present in the residues.

Algae: Considerable progress has been made in extracting and isolating sugars from an alga (Scenedesmus obliquus). It has been determined that the optimum hydrolytic procedure for setting sugars free from the cell material is to utilize 1 N H_2SO_4 in a boiling water bath for a period of 9 hours. This has produced hydrolysis to the extent of 11.5 per cent sugar. The adoption of an iodine-sugar determination method in place of the Benedict method previously used has made sugar determination in the hydrolysis more dependable.

Additional work has been carried out with 141 g (dry weight) of algae. The following methods were used:

1. The algae were extracted six times with hot 80 per cent alcohol and the extract distilled:

Insoluble fraction (vacuum dry).....	110.65 g
Residue from distribution of extract.....	38.20 g

2. The insoluble fraction was extracted with methanol followed by petroleum ether:

Insoluble fraction (vacuum dry).....	103.20 g
Removed by extractions.....	7.45 g

3. Again the insoluble fraction was hydrolyzed with 600 cc 1 N H_2SO_4 for 11 hours in a boiling bath, cooled, and centrifuged:

Nonhydrolyzable residue (actual weight)...	31.19 g
Material in hydrolysate (by difference).....	72.01 g

The sulfate was quantitatively precipitated from the supernatant liquid and the mixture was centrifuged. The supernatant liquid (3100 cc) contained 14.34 g of sugar (13.06 per cent of 103.20 g of cells). The material in the hydrolysate (72.01 g) contained 20 per cent sugar. Thus, removal of the nonhydrolyzable material from the dry cells, enriches the sample from 13 per cent to 20 per cent sugar content.

4. Two hundred forty-three g of moist Amberlite IRA-400 was added to the 3100 cc of liquid. The mixture was shaken occasionally throughout one day, filtered, and the sugar determined. The solution now

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contained only 5.07 g sugar (5.2 per cent of 103.20 g):

Not removed from hydrolysate by
Amberlite IRA-400 (actual weight)..... 24.70 g

(Sugar content of this was 5.07 g equal 20 per cent)

Removed by Amberlite IRA-400 (by difference) 47.31 g.

Many attempts to elute the material held by the Amberlite were made, but less than half could be eluted and none of the eluted material contained sugar. The Amberlite with the 47.31 g of material adsorbed was stored under water during warm weather, and it is possible that with several months storage the sugar was destroyed due to contact with the strongly basic Amberlite.

The experiments, summarized below, were carried out with the material not removed from the hydrolysate by Amberlite (24.70 g). The object of all the experiments was to increase the sugar content of the material.

1) Chemical reagents: Lead acetate, barium carbonate, and acetic acid were added to different portions of the water solution under different experimental conditions and followed by different procedures. No enrichment of sugar content was achieved.

2) Chromatographic columns: Seven adsorbents that have been used with success in sugar work were investigated: Fuller's earth; Florex xxx - a Fuller's earth type of adsorbent; calcium carbonate; silene EF; hydrated calcium acid silicate; Alumina; Magnesol; hydrated magnesium acid silicate; and silicic acid. The first four adsorbents were found to be ineffective. Alumina held the sugar preferentially, i.e., the first part of the effluent that came from the column was less rich in sugar than that which came later. However, it was difficult to elute the sugar from the Alumina. With silicic acid the first third of the effluent in one run showed 100 per cent enrichment. In other runs with different experimental conditions, the enrichment was definite but not so spectacular. The action of Magnesol was similar to that of silicic acid, with enrichment of the same order. There seems to be more soluble inorganic matter in Magnesol than in silicic acid. This is very difficult to remove completely by washing with water. It can be removed later, however, by extraction of the residues from the evaporation of the column effluent with 95 per cent alcohol. More work will be done with the study of silicic acid and Magnesol as reagents for sugar enrichment in solutions. They hold definite promise of usefulness but, so far, it has only been possible to push the enrichment from the original 20 per cent to about 28 to 30 per cent sugar content.

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3) Solvents: Having shown that enrichment could be increased to only about 30 per cent by chromatographic columns, efforts were next made to increase the sugar content further by the use of solvents. The different constituents in the samples should have different solubilities in organic solvents. By extraction of the dry sample with some of these solvents, a fractionation into soluble and insoluble portions with different sugar contents from that of the original sample, should be achieved. This proved to be the case. Only three solvents and combinations of solvents have been tried to date, but possibilities for the use of other solvents and combinations are almost unlimited. Many experiments with 95 per cent alcohol and absolute alcohol were carried out but only one with a combination of alcohol and ether, and this gave the most promising results. The results obtained in a few of these experiments are given in some detail in Table 14.

Table 14

RESULTS OF SOME EXPERIMENTS WITH SOLVENTS

Solvent	Weight (mg)	Sugar content (mg)	Sugar (%)
100 cc (Orig. sample	1355.0	384.5	28.3
Hot (Insol. part	Not studied		
95% alcohol ----- (Sol. part	794.0	277.0	34.9
25 cc (Orig. sample	1012.0	346.2	34.2
Cold ----- (Insol. part	310.0	102.4	33.0
Abs. alcohol (Sol. part	526.5	211.5	40.1
13 cc (Orig. sample	310.0	110.7	35.7
Cold ----- (Insol. part	205.0	68.8	33.5
Abs. alcohol (Sol. part	71.0	33.4	47.0
10 cc (Orig. sample	177.5	70.9	39.9
Abs. alcohol ----- (Insol. part	107.0	47.0	43.9
plus (Sol. part	61.0	19.0	31.2
75 cc ether			

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89BIOSYNTHESIS OF VITAMIN B₁₂, LABELLED WITH CO⁶⁰

In cooperation with Abbott Laboratories, preliminary work has been initiated on the problem of biosynthesizing Co⁶⁰-labelled vitamin B₁₂ in Streptomyces griseus. The necessary equipment for fermentation of the fungus was obtained and installed in a controlled-environment, plant growth-room. Fermentation of the cold process was initiated while awaiting the arrival of the isotope.

Fermentation products were used for the isolation process, following the Merck patent (April 2, 1948). At present work is in progress to obtain a satisfactory recovery of the vitamin and eventually to obtain the crystalline product.

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90Radiochemistry

Dr. William P. Norris
Mr. Walter Kisielewski
Dr. James S. Arnold
Dr. John Pomeroy
Mrs. Blanche Lawrence
Miss Lois Woodruff
Mr. Richard Sato

DETERMINATION OF COINCIDENCE ERRORS IN GEIGER TUBES

by
R. Sato and W. P. Norris

ABSTRACT

Careful determinations of the counting rate of a standard radioactive sample on five closely similar mica, end-window Geiger tubes over a period of several weeks have shown considerably more variation in count than expected. Inasmuch as the only variable that was not adequately controlled was the dead time of the Geiger tube itself, it seemed advisable to investigate resolution losses as a possible source of error.

The method used was an extension of that used by Kohman.⁽¹⁾ The radioactive source was composed of eight individual sources (Figure 13). The individual sources (which were essentially free of coincidence error) were counted alone and then together in combinations to give various desired counting rates. The true count, represented by the sum of the individual counts, was compared to the count obtained by counting the samples together.

The results indicate the dead time of our Geiger tubes* to be in the order of 7 to 8×10^{-4} seconds as compared with values of 2×10^{-4} seconds which are ordinarily considered to obtain. Coincidence errors are therefore about 6 per cent at a counting rate of 100 counts per second rather than the 2 per cent which would obtain with a resolving time of 2×10^{-4} seconds.

The importance of Geiger tube dead time to counting accuracy should be emphasized, especially since dead time may change slowly with time. A simplified method of determining dead time is being developed.

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*Manufactured by the Victoreen Company.

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PHOTOGRAPHED BY JANE K. GLASER

Figure 13. Sample holder used in the determination of coincidence errors in Geiger tubes.

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PROGRESS REPORT: EFFECTS OF X RADIATION ON SOLUTIONS
CONTAINING ORGANIC STABLE FREE RADICALS

by
J. H. Pomeroy

The effect of X irradiation upon solutions containing stable free radicals is being investigated. For instance, after irradiation a dilute solution of diphenyl picryl hydrazyl (DPPH) in chloroform loses its characteristic purple color as a result of the loss of the absorption band at $525\text{ m}\mu$. It is believed that this decolorization of the solution is caused by the combination of the DPPH radical with the free radicals formed from the solvent by the absorbed radiation.

The possible use of such solutions or gels of stable free radicals as chemical radiation dosimeters is under study. The use of such systems to trap the reactive fragments formed by radiation should also be of use in studying the chemical effects of radiation.

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PROGRESS REPORT: METHOD FOR EMBEDDING HARD MATERIALS

by

James S. Arnold

The principal goal of this problem is to develop a method for making detailed radioautographs of bone containing α - and β -emitting isotopes. This has involved checking various fixatives, embedding media, and cutting and staining techniques, as well as the actual autograph preparation.

Bones containing recently deposited Ca^{45} and P^{32} were fixed in 90 per cent alcohol or formol. In each case the fixatives were tested in three ways: 1) in the monol form, 2) buffered with borax, and 3) buffered with MgCO_3 . It was noted that the loss of either isotope to the fixative solution after 24 hours was negligibly small in alcohol; but as much as 10 per cent of the contained isotope was lost when formol was used. Preliminary results indicate that the buffering process did little to preserve the isotopes in bone.

At the present time we are trying out the freeze-drying technique of fixation that should be most satisfactory for histologic work.

In the course of the work it was recognized that the established methods of celloidin embedding were unsatisfactory from several stand-points. Penetration of the tissue is quite difficult in most cases. To improve penetration acetone has been substituted for the usual ether-alcohol mixture as a solvent. Preliminary results suggest that acetone used as a solvent for nitrocellulose, not only improves the penetration but provides more rapid and uniform drying and hardening.

It was also noted that well-hardened celloidin material lacked the adhesive and cohesive properties necessary for the cutting of dense hard materials, such as bone. For this reason it has been the practice of many workers to add a thin layer of celloidin to the cutting surface before cutting each section. In an effort to improve the adhesive and cohesive properties of the embedding medium, several plasticizers were tested. These included castor oil, Hercolyn D, and diamylphthalate. It was found that in acetone solutions the castor oil and Hercolyn D became clouded when drying in air. This was thought to be due to the hygroscopic tendency of the drying surfaces. Solutions using diamylphthalate were found not to cloud on drying. They had excellent cohesive and adhesive properties when used in 30 to 45 per cent (by weight) mixtures with the nitrocellulose. Preliminary results indicate this material may be a much superior embedding medium for cutting thin as well as thick sections of bone.

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It is well known that alkaline solutions are essential to prevent removal of heavy metal isotopes. Also treatment with salts of heavy metals is to be avoided because of the possibility of exchange reactions. Almost every method of staining reported in the literature requires the use of a heavy metal mordant or an acid medium. Upon investigation of coal tar dyes it was found that nuclear staining with safranin O and protoplasmic staining with azure could be obtained in alkaline alcoholic solutions.

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Biochemistry

Dr. E. S. Guzman Barron
Mrs. Veronica Bonzell
Miss Betty Gasvoda
Miss Louise Seki

THE EFFECT OF X IRRADIATION ON SOME PHYSICO-CHEMICAL
PROPERTIES OF PROTEINS

by

E. S. G. Barron, Veronica Bonzell, Betty Gasvoda, and
Louise Seki

ABSTRACT

It has been shown previously⁽¹⁾ that X irradiation produces destruction of the tyrosine residues of serum albumin, as measured spectrophotometrically.

Irradiation of serum albumin with doses of X rays ranging from 10,000 to 60,000 r failed to show any measurable changes in sedimentation rate or in electrophoretic mobility. Since we had previously observed protein denaturation and precipitation on irradiation with 70,000 r, it was decided to increase the dose to 100,000 r and study sedimentation rate, electrophoretic mobility, and viscosity.

The protein concentration was 1 per cent dissolved in 0.1 M phosphate buffers. Irradiation was performed with a 250-kv X-ray machine, at a temperature of about 25°C or at 6°C. In every case the nonirradiated sample was kept under identical conditions. The X-ray dose varied from 100,000 r to 200,000 r. The proteins were irradiated at their isoelectric point, at the acid side, and at the alkaline side of the isoelectric point. Crystalline bovine serum albumin was thus irradiated at pH 3, 4.47 (isoelectric point), 7, and 8.9. Gamma globulin (bovine) was irradiated at pH 3, 6 (isoelectric point), 7, and 8.9.

As far as the electrophoretic measurements were concerned 100,000 r caused no appreciable changes either in the surface area or in the mobility of the protein.

On measuring the sedimentation rate of albumin and globulin irradiated at pH 3.0, a second faster-moving component was observed in both proteins. Addition, previous to irradiation, of 6×10^{-3} M of cysteine prevented the formation of this second component. It seems as if this second component is a "dimer," i.e., a component made up of two protein

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molecules joined together by oxidation of their -SH groups: protein-S-S-protein. Cysteine would then prevent such oxidation.

Irradiation of the proteins at other pH values failed to produce the faster-moving component.

There were pronounced changes in viscosity after irradiation. Serum albumin irradiated at pH 3 showed a large increase in intrinsic viscosity, while globulin irradiated at the same pH value showed no change at all. When the proteins were irradiated at their isoelectric points, albumin showed no change in viscosity whereas the globulin viscosity increased by about 20 per cent. On irradiation at pH 7, there was 30 per cent increase in the viscosity of albumin, while the viscosity of globulin showed an appreciable decrease.

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THE RESPIRATION AND ACETATE OXIDATION RATE OF
X-IRRADIATED BACTERIA
(MYCOBACTERIUM CREATINOVORANS)

by

E. S. G. Barron, Veronica Bonzell, Betty Gasvoda, and
Louise Seki

ABSTRACT

It was shown previously⁽¹⁾ that X irradiation of Mycobacterium creatinovorans with doses varying from 1000 r to 10,000 r caused an increasing inhibition of respiration of the bacteria irradiated in the culture media containing acetate. On irradiation of three-times washed bacteria the residual respiration was unaffected by 6000 r while respiration in the presence of acetate was 30 per cent inhibited.

Many investigators have shown in experiments with animals (rats and drosophila) that the effect of X ray may be cumulative, i.e., small doses of X rays given continuously may produce similar effects to those produced on irradiation with a single large dose. To see if permanent changes could be produced in bacteria by continuous irradiation with relatively small doses of X rays, liquid cultures of M. creatinovorans (in acetate medium) were irradiated with 1000 r. One-day-old cultures were irradiated in test tubes with 1000 r (effective irradiation about 800 r); the cultures were transplanted 8 hours later into another test tube which was irradiated on the following morning. These successive irradiations started on July 18, 1949, and ended on August 11, 1949. The total amount of effective radiation was 11,200 r. The cultures were transferred twice weekly from August 11 until October 10, 1949, when the respiration (residual and in the presence of acetate) was measured and was compared with that of the control nonirradiated samples. As can be seen in Figure 14, the residual respiration, as well as the oxidation rate of acetate, was depressed in the X-irradiated bacteria. Pigment formation in the X-irradiated bacteria was also less than in the control samples.

The X-ray dose received daily, 800 r, when given in a single dose had no effect either on multiplication of bacteria or on respiration.

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8-11-49-97

Figure 14. Bacteria irradiated with 1000 r five days a week from July 18 to August 11, 1949. Transferred twice weekly until October 10, 1949.

1. X-irradiated bacteria; no substrate
2. X-irradiated bacteria; acetate
3. Control; no substrate
4. Control; acetate pH, 7.0; temperature 38°.

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Microbiology

Dr. E. Lawrence Powers
Dr. C. S. Bachofer, C.S.C.
Mr. Charles F. Ehret
Mrs. Sylvia D. Mayer
Mrs. Carlene M. Raper
Mrs. Deborah Shefner

PROGRESS REPORT: PHYSIOLOGICAL STUDIES ON THE MATING
REACTION IN PARAMECIUM BURSARIA. I. SOME INDICATIONS
FOR AN ACTION SPECTRUM IN THE MATING REACTION

by
C. F. Ehret

The agglutination phase of the mating reaction of Paramecium bursaria occurs with a periodicity that is light sensitive. The periodicity is diurnal when cultures are illuminated for arbitrary fixed periods during regular 24-hour intervals; diurnal periodicity of cultures pretreated in this manner persists indefinitely in the dark. Ten-hour light periods (with 14-hour dark periods) have predominantly been employed in these investigations. When cultures are exposed to the unfiltered irradiances from 30 to 40 W daylight fluorescent units, the mating reaction magnitude (RM)-agglutination phase is increased early in the light period, but reduced later. Thus from 1 to 3 hours before the dark period, nonirradiated cultures give a larger RM than those irradiated with light of the visible spectrum as obtainable from the indicated sources.⁽¹⁾

Recent experiments indicate that the inhibition that is seen with the fluorescent source late in the light period does not occur when the organisms are irradiated with equivalent energies between 350 and 500 m μ . (The transmission peak for the CuCl₂ cell + glass filter combination used was at 450 m μ .) In addition to the failure of this blue-violet light to inhibit during the late light period, a definite increase in RM occurred, which ranged from 15 to 50 per cent greater than that of the nonirradiated controls.

In contrast to the RM inhibition that the longer wave-lengths cause late in the light period, during the early hours all spectral regions investigated caused an increase of at least 20 per cent in RM. Included were spectral regions obtained with CuCl₂ (long wave-length cut-offs* at 650 m μ) plus glass filters with the following short wave-length cut-offs: 460, 500, 580, 635 m μ . The blue-violet region was again the most effective, causing an RM increase about 50 per cent greater than that caused by equivalent energies at the longer wave-lengths of the different spectral regions

* Below the cut-off, <1 per cent transmission.

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employed. A more critical evaluation of relative efficiencies will be available upon completion of a determination of the action spectrum with monochromatic light.

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PROGRESS REPORT: PHYSIOLOGICAL STUDIES ON THE MATING
REACTION IN PARAMECIUM BURSARIA. II. EFFECT OF X RAYS
ON THE MATING REACTION

by
C. F. Ehret

The effect of X rays on the mating reaction in P. bursaria has been investigated in an essentially qualitative manner by Wichterman,⁽¹⁾ who reports that in the dose range investigated (100,000 to 1,000,000 r) the agglutination clumps formed are smaller than in nonirradiated cultures, and that above 400,000 r, there is little or no mating reaction directly after irradiation.

Recent experiments in this laboratory confirm the inhibitory effect of X irradiation on the RM, but in a lower dose range, from 10,000 to 40,000 r (100 kv, 5 ma, and 0.25 mm Al added filtration). In this range, there is a dose-dependent decrease in the RM; at 10,000 r the value is equal (in the mean of 13 experiments) to the mean of the nonirradiated controls, and the RM at 40,000 r is equal to 8 per cent of the control. However, below 10,000 r, a stimulatory effect has been observed. In the mean of 13 experiments, this effect is proportional to the dose, with a maximum at 8000 r, at which the RM is 235 per cent that of the nonirradiated controls. As in the case of blue-violet light, this type of response appears only during the light period. No reactions have been observed to occur in either X-irradiated or blue-violet light-irradiated mixtures during the dark period.

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Radiobiochemistry

Dr. Jack Schubert
 Dr. Marcia R. White
 Dr. Lawrence S. Myers
 Dr. Arthur Lindenbaum
 Mr. Roman Lesko
 Mr. Herschel Wallace
 Miss Jean Jackson

PROGRESS REPORT: RADIOBIOCHEMISTRY

by
 J. Schubert and Marcia R. White

COMPLEX ION STUDIES

Studies of the association constants of complex ions formed between Ca^{++} and Sr^{++} and organic acids are continuing. These are useful in interpretation of the metabolic behavior of these elements in the body. The general method and procedures employed were described in the previous quarterly report. The present measurements were made in isotonic NaCl buffered with veronal, pH = 7.3, and tracer levels only of Ca^{45} and $\text{Sr}^{89,90}$.

Acid	Association constant, K_a	
	Ca	Sr
Citric	2360.	590
Tricarballic	66.2	48
Tartaric	60.6	35
Succinic	10.4	5.6
Malic	116	28
Malonic	22.7	19.2
Maleic	14.2	13.1
Citraconic	20.2	--

TREATMENT OF RADIATION SICKNESS

Radiation sickness is the result of complicated chains of biochemical reactions resulting from the radiation-induced ionizations. A series of compounds, each of which is known to act upon specific stages in certain metabolic processes, has been selected for injection into irradiated animals. It is hoped that by their interference in certain biochemical

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processes these compounds will inhibit or enhance radiation effects. The mechanisms involved in radiation sickness may thus be clarified and rational means of treatment developed.

Each compound used was given in a concentration designed to give physiologic but not toxic effects. In each experiment 18 to 20 rats were exposed to a single, total-body exposure of X rays in groups of 10. The radiation factors were 200 kv; 15 ma; 0.5-mm Cu and 3.0-mm Bachelite filters; 28.5 inches target distance; and dose rate of about 16 r per min. Six rats were treated before radiation (from a few minutes to 2 hours); six after radiation (15 minutes or less); and the rest served as controls.

Table 15 summarizes the results to date.

Table 15

Compound	Route of application	Dosage of X ray	Average time of death (days after X ray)		
			Controls	Pre-treated	Post-treated
Copper gluconate	S.C.	800 r	9.9	8.5	(11.5)*
Copper gluconate	I.V.	900 r	5.2	6.0	5.6
Sodium orthovanadate	I.V.	900 r	5.2	5.4	5.5
Sodium fluoroacetate	I.P.	900 r	5.8	6.5	6.4
Malononitrile	I.V.	900 r	6.0	7.0	6.3†
Sodium iodoacetate	I.V.	900 r	6.5	4.9	5.1
Disodium malonate	I.V.	950 r	5.0	5.5	5.2
Manganous gluconate	I.V.	950 r	5.3	4.4	4.7

*Average of 5 rats only. The sixth was still living 58 days after irradiation.

†Post-treated animals were given multiple injections of malononitrile up to 7 days after irradiation.

TREATMENT OF RADIOELEMENT POISONING

Plutonium: The following summarizes recent studies of the effect of zirconium on the metabolism of Pu. The techniques used are the same as those previously described. A more detailed report is being prepared.

The distribution of Pu at three days is independent of the route of injection (I.V. or I.P.) in the femurs, liver, kidneys, lungs, heart, blood, and probably the spleen, in both rats and mice. Following I.P. injection more Pu is in the pancreas, mesenteric lymph node, and gastro-intestinal tract than after I.V. injection. In rats, the pancreas has 39 times, the node

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3.7 times, the G.-I. tract 2.4 times as much Pu after I.P. injection than after I.V. injection, while the corresponding figures for mice are 6, 1.6, and 2.8.

The percentage distribution of Pu in the tissues of rats and mice is very similar. In the mouse less Pu is in the femurs and spleen and more in the liver than in the corresponding tissues of the rat.

The effect of different dose levels of intravenously injected Zr citrate (from 5.1 to 99.0 mg of Zr per kg) given 30 minutes after I.V. injection of Pu on the tissue distribution of Pu in rats at three days was as follows:

- 1) The bone content of Pu following dosages of Zr 13.1 mg per kg was reduced to approximately $1/2$ the control level, but was unaffected by 5.1 mg per kg.
- 2) The liver content of Pu was unchanged after low or high doses of Zr, but was increased from a control level of 31.2 to 46.0 and 40.4 per cent of the injected dose after 13.1 and 26.1 mg per kg of Zr, respectively.
- 3) The spleen content of Pu is increased almost $2\ 1/2$ -fold after 5.1 mg per kg of Zr, but decreased proportionately following increasingly higher dosages.
- 4) The kidney content of Pu is decreased by about $1/2$ following 5.1 and 99.0 mg per kg of Zr, but is unaffected by intermediate dosages.

The effect of different dosages of Zr citrate (from 12.8 to 112 mg per kg as Zr injected I.V.; or from 116 to 871 mg per kg as Zr injected I.P.) given 30 minutes after I.V. injection of Pu on the tissue distribution of Pu at three days in the mouse is as follows:

- 1) The bone content of Pu is reduced to from $1/2$ to $3/4$ of the control level. The amount of reduction is independent of dosage and route of administration of Zr.
- 2) The liver content of Pu is unchanged following the I.V. injection of 12.8 mg per kg of Zr, but is lower following the large dosages, whether given I.V. or I.P. The degree of reduction is very roughly proportional to the dosage, with a maximum reduction to 60 per cent of the control level.
- 3) Spleen content of Pu is less than 0.2 per cent of the injected dose in controls and all Zr treated mice.

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- 4) Kidney content of Pu is reduced from 33 to 70 per cent of the control level, the degree of reduction being roughly proportional to the dosage. The Zr given I.V. may be more effective than that given I.P.

A colloidal solution of Zr citrate is more effective than the non-colloidal solution used in the preceding experiments in reducing bone and liver deposition of Pu. Intravenous injection of 72.3 mg per kg of Zr in a colloidal solution 30 minutes after I.V. injection of Pu reduces bone content by 84 per cent at three days, i.e., to the 30 minute control level. The liver content of Pu is reduced 40 per cent.

Either colloidal or noncolloidal Zr injected I.V. 30 minutes after I.V. injection of Pu reduces the blood content of Pu 42 per cent of the control level (from 55.5 per cent to 23 per cent of the injected dose) in 5 minutes, to 20 per cent in 30 minutes, and to 13 per cent in 90 minutes.

Most of the Pu retained by a dog 1330 days after I.V. injection of 0.423 mg of Pu (28.8×10^6 cpm) as Pu (+4) nitrate was retained in the skeleton (about 83.4 per cent of the injected dose) and the liver (7.6 per cent of the injected dose). All other tissues contained very small amounts.

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Neutron Toxicity ProgramDr. Raymond E. Zirkle
Dr. Howard H. Vogel, Jr.PRELIMINARY REPORT: A QUANTITATIVE TEST OF RADIO-
BIOLOGICAL ADDITIVITY OF FAST NEUTRONS AND
GAMMA RAYS

by

H. H. Vogel, Jr., R. E. Zirkle, and A. M. Brues

The first experiments planned for the neutron program have been devised to test the radiobiological additivity of fast neutrons and gamma rays in their acute lethal action on mice.

Zirkle *et al.*⁽¹⁾ have reported that fast neutrons and gamma rays are completely additive in their acute lethal action on CF-1 mice. Gray and Read⁽²⁾ published results of similar experiments on the root of the broad bean and also found that within the experimental error the lethal actions of fast neutrons and gamma rays were completely additive. However, Mitchell⁽³⁾, using these same radiations to determine the 21-day LD₅₀ in mice, observed incomplete additivity. His doses were delivered in 24 or 48 hours, whereas in Zirkle's work the doses were delivered in about one hour. Unfortunately, because the nature of the available equipment necessitated the irradiation of individual mice, Mitchell's results are based on small numbers of animals. Zirkle⁽⁴⁾ has recently summarized our present knowledge of radiobiological additivity of various ionizing radiations.

We plan to test the additivity of the two radiations at widely different dose rates, since the meager literature now available indicates that the dose rate may influence the results. We plan specifically to use dose rates that will deliver the 30-day LD₅₀ in approximately 1 hour, 6 hours, and 24 hours. CF-1 mice will be used.

For each of the three proposed dose rates we plan to obtain data for three survival curves: one for fast neutrons, one for γ rays, and one for the two radiations combined in approximately equal radiobiological intensity. Thus, nine survival curves will be obtained in all. To establish each survival curve, we plan to use 10 graded doses with at least 100 mice exposed to each dose. This means that at least 9000 mice will be used in these experiments. We anticipate that approximately half the animals will survive the period of acute mortality and will be available for longevity studies.

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The exposures will be made on top of a reactor. The source of fast neutrons will be a converter consisting of a horizontal layer of natural uranium about 1 inch thick and 30 inches square. This, when in use, will be centered on the vertical axis of the thermal column. The animals will be placed in a single layer about 12 inches square at a height of 5 or 6 inches above the converter. Suitable shields will be interposed to prevent γ rays and slow neutrons from impinging upon the mice. The dosage rate of fast neutrons will be measured with 100 r chambers of Victoreen condenser r-meters, and will be expressed in n-units. We expect to be able to irradiate at least 50 mice simultaneously in the animal chamber. When not in use, the converter will be housed in lead adjacent to the thermal column.

Since the γ rays produced with fission cannot be measured satisfactorily in the presence of fast neutrons and, moreover, are not of sufficient radiobiological intensity to match the effectiveness of the neutrons, we propose to use γ sources of Co^{60} . When in use, these sources will be in a circle above the animal chamber, whether or not the animals are being simultaneously irradiated with fast neutrons from below. When in use, the γ sources will be encased in local shields of lead and will be stored, when not in use, in a lead housing directly to one side of the thermal column. The intensity of the γ radiation reaching the animals will be increased or decreased by adjusting the distance of the Co^{60} source from the animals and by using sources of different activities. The dosage rate of the γ rays will be measured in roentgens with a Victoreen r-meter.

Thus the mice can be exposed to γ rays alone (with the pile not in operation); they can be exposed solely to fast neutrons from the uranium converter (with the pile in operation, but the Co^{60} source removed from its position above the animal chamber); or, the mice can be irradiated simultaneously with γ rays from above and fast neutrons from below. Using each of these three methods of irradiation, at the three proposed dose levels, we should be able to determine whether the radiobiological additivity of fast neutrons and γ rays is complete or not in their lethal action on CF-1 mice. The results of these experiments should have both theoretical and practical significance in our understanding of the biological effects of ionizing radiations. Implementation of this program is dependent upon suitable alterations being made to the reactor thermal column and the adjacent experimental area.

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Miscellaneous

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FECAL AND URINARY RESPONSE TO CHOLERA VACCINE IN
X-IRRADIATED GUINEA PIGS

by

W. Burrows, Norma G. Deupree, and Dorothy E. Moore

ABSTRACT

The effect of pre- and postimmunization X irradiation in dosages ranging from 50 to 200 r on the immune response of guinea pigs to cholera O vaccine was assayed as serum, fecal, and urinary antibody (agglutinin). X irradiation following immunization did not appreciably affect serum antibody titer, but dosages of 100 r and 150 r partially or completely inhibited the excretion of fecal and urinary antibody. Excretion could be partially restored by booster inoculation. Preimmunization irradiation sharply reduced serum antibody titer, but produced a transitory stimulus of fecal and urinary antibody, apparent when immunization was begun at one day, but not 3 days, after X irradiation. #

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EXPERIMENTAL ENTERIC CHOLERA IN THE X-IRRADIATED
GUINEA PIG

by

W. Burrows, Norma G. Deupree, and Dorothy E. Moore

ABSTRACT

The effect of X irradiation in single total-body dosages of 100 r or 200 r on enteric cholera in guinea pigs was assayed by autopsy culture of animals sacrificed at intervals after irradiation and by the number of vibrios and titer of antibody excreted in the feces. In the 63 per cent of the animals that died after exposure to 150 r, death was associated with generalized bacterial infection. Cholera vibrios were found in 44 per cent of these animals and hemolytic streptococci and staphylococci were found in the remainder. Thus, at this dosage there resulted a generalized tissue invasion of cholera vibrio from a focus of infection in the bowel which was not found in infected, nonirradiated animals. With dosages of 150 r and 200 r the intensity of the infection was reduced during the first three days following inoculation concurrent with that of antibody response, but subsequently the antibody titer declined and the infection increased. *Saps of 100 r* While the infection was appreciably reduced in intensity in its early stages by dosages of 100 r, coproantibody response was not effectively inhibited, and both intensity and duration of the infection were reduced compared with that in the nonirradiated animal. *By do-* The inverse relation between coproantibody titer and the intensity and tendency to generalization of the enteric infection provides additional support for the hypothesis that effective immunity to enteric infection is in part a function of coproantibody. If it is assumed that natural immunity to bacteria of intestinal flora is also related to coproantibody response, the inhibition of this immune response by lethal dosages of X radiation may account for bacteremia of intestinal origin in irradiation sickness.

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THE EFFECT OF TRITIUM (H^3) ON CELLS CULTIVATED IN VITRO

by

Agnes N. Stroud and A. M. Brues

INTRODUCTION

A preceding preliminary report⁽¹⁾ dealt with some effects of tritium on 10- to 11-day chick embryo muscle fibroblasts. Further studies have been made of mitosis, chromosome behavior, and radiosensitivity of cells exposed to this soft β radiation, which has a range of about 2 to 3 μ in water or tissue.

Tritium offers a source of continuous radiation exposure to the cells. When it is incorporated into the nutrient medium as H_2^3O , it is possible to study long-term effects of irradiation under relatively constant conditions and without radiation exposure to the experimenter.

It has been well established by Canti⁽²⁾, Spear⁽³⁾, Lasnitzki⁽⁴⁾, Cox⁽⁵⁾, Paterson⁽⁶⁾ and others, that with γ rays, X rays, radium, and hard β rays, there are radiation effects of two kinds on cells in tissue culture: temporary or reversible, and permanent or irreversible. Following single or fractionated dosage, mitosis is suppressed partially or completely, followed by recovery after a few hours. The effects of continuous exposure are less well understood, but it is known that recovery is incomplete.

Several points of interest can be settled by a careful quantitative study of mitotic responses to continuous irradiation at different dosage rates. Analysis of cultures treated at different dosage rates should enable a recovery factor to be calculated in a way quite different from that employed in making such calculations by observation of recovery after single doses; and delay of division observed under irradiation may be useful in evaluating mitotic counts on tissues receiving internal irradiation. Also we have used cinematography on stained preparations in conjunction in an attempt to understand better the mechanisms whereby cell division is inhibited, delayed, or distorted.

An attempt has been made to determine at which mitotic stage inhibition or blocking of mitosis occurs. Almost every phase has been named in the literature as the sensitive stage. Cinematographic records show that cell death resulting from continuous irradiation up to a few thousand reps, does not occur immediately but during or following mitosis and that death may occur by degeneration during an unsuccessful attempt at mitosis. When much higher doses (e.g., 50,000 rep and above) are administered to a culture many resting cells explode, extruding cytoplasmic granules, and the nucleus immediately shrinks and becomes angular (Figure 15A). This

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A. Exploded cell showing shrinking of nucleus. The cell received 18,000 rep.

B. Multiple anaphase bridge as the result of 2000 rep at a dose rate of 62 rep/hour.

C. Chromosomes, short and thick, clumped at metaphase with a dicentric ring chromosome. The cell accumulated a dose of 8700 rep at 167 rep/hour.

D. Chromosomes in cluster formation at a dose of 920 rep given at 167 rep/hour.

E. Cell in telophase showing spindle suppression and depletion of nucleic acid (heterochromatin) and "stickiness" of chromosomes on receiving a dose of 8700 rep at 167 rep/hour.

F. Polyploid cell with incomplete multipolar spindles 2000 rep/hour.

G. A dose of 2000 rep at 62 rep/hour was received by the cell. Late telophase showing an acentric fragment and nucleic acid probably streaming along spindle fiber path (possibly chromosome fragments).

H. Degenerate cell showing the deposition of chromatin around vacuoles. Two thousand rep at a dose rate of 42 rep/hour.

Figure 15.

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phenomenon is characteristic of intermitotic cells in cultures so treated that very few enter mitosis. The cells explode in a random fashion, and the relation of this effect to the mitotic anomalies is not clear.

MATERIALS AND METHODS

Cultures of chick embryonic skeletal muscle fibroblasts taken from 9- to 11-day embryos were prepared by either the coverglass hanging drop method or by the Gey lying drop technique for phase microscopy. The nutrient medium consisted of Tyrode's salt solution, and the tritium water was incorporated into this medium. The hanging drop preparations were incubated for 24 hours at 37°C and then examined under the microscope and placed in groups of approximately equal mitotic activity and growth radius before they were treated with tritium-containing medium. After treatment, they were returned to the incubator for varying lengths of time (until they accumulated the desired exposure dose), after which they were fixed in Bouin's solution and stained with Feulgen stain.

In the stained preparations mitotic figures and resting cells were counted and classified as prophase, metaphase, anaphase, and telophase. A count was also made of the spireme forms and the number of degenerate and exploding cells. The areas of the colony used for counting of cells in the treated and control cultures were at the zone of densest migration (zone III of Mayer⁽⁷⁾), which represented an area of about 1.8 sq. mm. In each preparation 1000 cells were counted, and the number of normal and abnormal mitotic figures was recorded. Counts in one experiment carried out at a very low dose rate were made on the number of cells per microscopic field using the entire peripheral zones III and IV of Mayer.⁽⁷⁾

The dosages used for treating the cultures ranged from 1000 rep per day to 72,000 rep per day (41.5-3000 rep per hour).

Continuous observation of immediate and delayed effects was made cinematographically using Cooke, Troughton, and Simms phase contrast microscope equipment with a 16-mm Cine-Kodak special camera adapted by George Svihla for time-lapse motion picture, operating at 6 frames per minute. A few minutes after receiving a radioactive medium, a cell in a particular stage of mitosis was selected for filming, and the duration of this phase and succeeding stages was timed visually. The length of time a cell remained in mitosis, as well as the duration of each phase, was measured.

Radiation dosage rates in the cinematographic study were 62, 83, 167, and 3000 rep per hour.

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OBSERVATIONS AND RESULTS

Chromosome abnormalities in cultivated tissues have been described by other investigators as physiological effects of irradiation. Some of the effects upon the chromosomes seen in the stained preparations are clumping, lagging at anaphase (Figure 15B), scattering (due to spindle suppression), "stickiness" seen at late metaphase or anaphase (Figure 15C), cluster arrangement at late prophase or early metaphase (Figure 15D), breaks, bridges, and evidence of nucleic acid depletion (Figure 15E). The most striking is the high percentage of abnormal mitotic cells showing clumped chromosomes. Chromosomes become clumped in an amorphous mass, which has been attributed to changes in viscosity and to alterations in the nucleic acid content. Rugh⁽⁸⁾ describes these same phenomena following X irradiation of Triturus pyrrhogaster, in which the chromosomes become less viscous, more fluid, and "sticky," i.e., clumped. Over 50 per cent of the cells showed clumped chromosomes after they received 200 rep, and this figure remained almost constant up to 1300 rep and at 2 different dosage rates. Foreshortening and thickening of the chromosomes occurred simultaneously in about 1/2 of these cells. Cinematographic records showed this same condition with the chromosomes clumped together in a cluster and rotating around a central axis for abnormally long periods depending upon the intensity and accumulated dose. This indicated a blocking effect that is similar to the effect of colchicine. Nucleic acid starvation (inferred from depletion in Feulgen-staining material), breaks, and bridges were seen only at higher dosages (500 rep and above).

Although cinematographic records suggested (on the basis of total cell morphology) that the delay or blocking occurred at metaphase, this is not entirely borne out by stained specimens, which showed a significant but slight suppression of prophases under certain conditions. It appears from counting that delays in mitosis may occur throughout an abnormal cycle and be seen, therefore, in various stages or modifications of classical stages. No particular "radiosensitive phase" was found, such as occurs after the administration of colchicine-like mitotic poisons.

It is known that after the breakdown of the nuclear membrane, when the chromosomes are approaching their maximum prophase development, spindle formation occurs. Following irradiation, spindle formation is upset and the failure of spindle attachment by "starvation" of the heterochromatin from loss of its nucleic acid (Darlington⁽⁹⁾, Caspersson⁽¹⁰⁾, Heitz⁽¹¹⁾, Darlington and LaCour⁽¹²⁾) causes the chromosomes to cluster and clump and remain in a semi pro- or metaphase condition for abnormally long periods of time. This was observed to occur cinematographically before anaphase took place. The chromosomes sometimes failed to line up at the equatorial plate, and there was usually unequal distribution of chromosomes (Figure 15F). With the higher intensities and doses employed, the effects were more pronounced and cells were seen to pinch off at anaphase

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unequally, or to degenerate at this stage ("pseudoamitosis"). The number of degenerate cells increased with increasing dosages. Cox⁽¹³⁾ believes that cell degeneration following X irradiation is correlated with cell division, and that the period of greatest radiosensitivity is indicated by cell degeneration. Lasnitzki⁽¹⁴⁾ has found in experiments with X rays on tissue cultures that degeneration produced by doses from 100 to 1000 r is mainly the result of unsuccessful mitosis. It should be possible, employing widely varying dose rates, to settle some question regarding the period of radiosensitivity.

Cytoplasmic as well as nuclear changes followed irradiation with tritium. Cinematographs showed clumping of mitochondria, change in viscosity, and increase in surface activity and activity of the cytoplasmic granules during exposure, but as the accumulated dose became larger there was a decrease in surface activity and clear protoplasmic protrusions (small blebs) appeared around the entire cell membrane.

Analyses of the total number of mitoses and of the separate phases, which were based on counts of 3000 cells and compared with controls, showed that at a low dosage rate (167 rep per hour) the "mitotic index" was not decreased more than one-third after 500 rep. At the higher dosage rate the index stabilized at about one-third of the control figure at 500 rep and remained there, even until 1300 rep had been received. In both cases prophases and anaphases were relatively fewer than metaphases, probably due to a tendency of delay to occur during metaphase. At lower dosages the proportion of cells passing through mitosis, including all phases, was increased over the controls, suggesting that prolongation of mitosis occurs first. (An actual increase in mitotic activity is not ruled out, however.)

The highest percentage of abnormal mitotic cells was in metaphase. Using a relatively high dosage rate (417 rep per hour) the percentage of abnormals remained about constant between 200 and 1300 rep accumulated dose. At a lower rate, however (167 rep per hour) a constant level of abnormal figures was not reached for 5 1/2 hours. This suggests the possibility that at the lower dosage rate cells were passing through one division and manifesting abnormalities of mitosis in the succeeding division.

DISCUSSION

Our findings on tritium-treated cultures are consistent with its radioactivity. With increasingly high doses and intensity there is a decrease in mitotic activity with a subsequent increase in the per cent of abnormal mitosis and the number of degenerate cells. There is both inhibition and delay of cell division, the duration of mitosis becoming at least three hours, and rarely is the mitotic division normal.

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"Nucleic acid starvation" has been seen in the treated cultures as evidenced by the understaining of chromosomes, and this may be related to lagging of chromosomes at anaphase, or loss of spindle attachments; other abnormalities include fragmentation or malposition of chromosomes and formation of bridges, from which chromosomes or fragments may slip apart or break off. Using continuous irradiation it is difficult to distinguish "physiological changes" from those which may follow structural damage to chromosomes.⁽¹⁵⁾ The interpretation of a chromosome bridge, for example, being a case in point (Figure 15G). Among the definitely physiological effects are those presumably attributable to anomalies in nucleic acid metabolism. For example, not all cells at all phases take up the Feulgen stain uniformly for that particular phase, although the controls stain very consistently at each stage. Suppression of the spindle is also clearly "physiological," whether or not attributable to nucleic acid deficiency. We have observed by motion picture that these cells can divide without lining up at the equatorial plate, in contradistinction to colchicine mitosis.

The activity of the cytoplasmic granules is suddenly increased with doses as high as 1500 rep probably due to viscosity changes; but the motion becomes sluggish at higher dosages. The resting cells seen in motion pictures explode after receiving 20,000 rep or more, extruding cytoplasmic granules; almost immediately the nucleus shrinks. The shrinkage of the nucleus also occurs in nonexploding dying cells. Feulgen-stained preparations of cells thus degenerating show the accumulation of deeply stained chromatin material around the nuclear membrane and vacuoles of exploded cells (Figure 15H).

The results of continuous irradiation with H_2O show that delay in completing mitosis may be due to a variety of immediate effects, including various physiological effects on spindle and chromosomes as well as chromosome breaks. This is suggested by the fact that even several hours after beginning irradiation, occasional mitoses are completed normally and without delay, and that motion pictures show delay occurring with several characteristic types of abnormal cell behavior. Since irradiation was continuous in these experiments, these variations are difficult to explain, as they might be in experiments involving after-effects of single irradiations, by differences in sensitivity of cells at different stages in the mitotic cycle. It appears more likely that mitotic delay occurs as the result of various random events resulting from the radiation, perhaps on the basis of abnormalities in nucleic acid metabolism.

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