ACUTE MORTALITY OF CHINCHILLAS EXPOSED TO MIXED GAMMA-NEUTRON RADIATIONS OR 250 KVP X RAYS

T. A. STRIKE
L. J. SEIGNEUR

R. E. GEORGE
Commander, MSC, USN
Chairman
Radiation Biology Department

HUGH B. MITCHELL
Colonel, USAF, MC
Director

ARMED FORCES RADIOBIOLOBY RESEARCH INSTITUTE
Defense Atomic Support Agency
Bethesda, Maryland

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FOREWORD
(Nontechnical summary)

The acute mortality of chinchillas exposed to ionizing radiation was studied at the Armed Forces Radiobiology Research Institute (AFRRI) as part of an effort to characterize the biological effect of mixed gamma-neutron radiations from the AFRRI-TRIGA reactor.

Chinchillas were exposed to the mixed gamma-neutron radiations or to 250 kVp x rays at selected doses throughout the lethal range (that range of doses resulting in death of from 1 to 99 percent of the exposed animals within 30 days). In addition, some animals were irradiated at higher doses to obtain survival time data in the dose range expected to cause 100 percent mortality within 10 days. All exposures were whole body, unilateral, and delivered at approximately 20 rads/min. Midline tissue doses ranged from 200 to 639 rads for mixed gamma-neutron radiations and from 357 to 1786 rads for x rays.

Chinchilla deaths were recorded daily and the resulting data subjected to mortality and survival time analysis. A statistical method (probit analysis) was used to obtain dose-mortality response curves and associated parameters including the median lethal dose (that dose which will kill 50 percent of the animals in a large group).

In this study the 30-day median lethal doses ($LD_{50/30}$) for the mixed gamma-neutron radiations and for 250 kVp x rays were calculated to be 295 and 490 rads, respectively. By comparing the $LD_{50/30}$ values, the RBE (Relative Biological Effectiveness) of the reactor radiations was found to be 1.7.

In previous radiation lethality studies, survival time ... Spragu--Dawley rats was reduced from approximately 10 days to less than 5 days by increasing the dose from the lethal range to about twice the $LD_{50/30}$. This result was not obtained when chinchillas were similarly treated.

ABSTRACT

The chinchilla's acute mortality response to mixed gamma-neutron radiations of the AFRRI-TRIGA reactor and to 250 kVp x rays was studied. Unilateral whole body irradiations were accomplished at doses from 200 to 639 rads of mixed gamma-neutron radiations and from 357 to 1786 rads of x rays. All radiations were delivered at approximately 20 rads/min, and doses are reported as midline tissue doses. The $LD_{50/30}$ values calculated for the mixed gamma-neutron radiations and for the x rays were 295 and 490 rads, respectively. Using 30-day median lethality as the end point, the RBE of the mixed gamma-neutron radiations was 1.7. The wide lethal dose range obtained was attributed to a high degree of variation in age of the chinchillas. In contrast to previous experience in rodents, the chinchilla showed a relative resistance to the classically described gastrointestinal modality of radiation death.

I. INTRODUCTION

A comparison study was initiated among several mammalian species to assess the biological effectiveness of mixed gamma-neutron radiations from the AFRRI-TRIGA Mark F reactor. The reference radiation was 250 kVp x rays, and the 30-day median lethal dose ($LD_{50/30}$) was selected as the biological end point for comparison. In the course of this research, an opportunity arose to study the response of chinchillas to ionizing radiations.

The chinchilla has many unique anatomical and physiological characteristics, therefore, its mortality response to ionizing radiations seemed especially worthy of study. This rodent has a 28-day estrous cycle and a 111-day gestation period,[12] a 12- to 20-year lifespan,[7] fine hair that can be painlessly plucked with as many as 50-60 hairs exiting from each hair follicle,[25] and a metabolism which results in odorless urine and feces.[14]

The mortality results of chinchilla exposures to mixed gamma-neutron radiations from the TRIGA reactor or x rays are the subject of this report.

II. MATERIALS AND METHODS

Adult chinchillas* of aniger strain were collected locally† from ranches throughout the United States. After a minimum conditioning period of 12 weeks, the chinchillas were transferred to environment-controlled animal rooms at this laboratory. They were individually caged and acclimatized an additional 4 weeks before

* These chinchillas represent stock discarded by the breeders because of their "fur chewing" tendencies. In all other respects these animals were normal and healthy.

† National Chinchilla Breeders of America, Inc., Chinchilla Industry Testing Center, Bethesda, Maryland

being irradiated. During this period, the chinchillas were weighed twice weekly and those animals exhibiting weight loss or symptoms of disease were excluded from the study. Food and water were available ad libitum. The diet consisted of guinea pig chow* and rough clover hay, supplemented twice weekly with apples.

In the initial series of exposures, a total of 512 chinchillas was used (Table I). Animals were assigned to dose groups in a random fashion, biased only by the fact that each group of 32 was equally divided as to sex. To test the reproducibility of the initial results, an additional 229 chinchillas were grouped randomly (16 or 20 animals

Table I. Chinchilla Mortality Data

MIDLINE TISSUE DOSE (RADS)*	DIED / EXPOSED	POSTIRRADIATION DAY OF DEATH** 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	PERCENT MORTALITY (30 DAYS)	SURVIVAL TIME (DAYS) MEAN	MEDIAN
X RAYS																																		
[illegible]	0 32																															0		
[illegible]	0 16																															0		
[illegible]	3 16									1					1								1									19	15.0	16.0
447	16 32										6			1	1			2	2	1	1		1						2	1		50	17.7	17.5
536	17 32			1		1	1		1	3					5	2	3		1	1	1											53	12.5	14.0
[illegible]	10 15							1		1	2	1		3		2																67	11.6	12.6
625	24 32					1	4	3	2			2	1	3	1	1			1	3								1			1	75	13.4	12.5
670	21 32				1	1		3	8			1	3	1		1	1				1		3							1		66	12.1	11.0
714	25 32					1	2			1	3	3	1	6	3	3			1	1				1		1						78	13.0	12.0
[illegible]	14 16								3	2		1	3	3		3											1					88	12.5	12.[illegible]
759	24 32	1				1	1		1	2	[illegible]	3		2	2	3	3				1								1			75	12.5	12.0
804	29 32						[illegible]	1	1	3	1	4	4	4	3	2	1		1				1									91	11.8	12.0
848	31 32						2	1	3	5	2	5	0	9		3																97	10.6	11.0
893	27 32					1	1	2	3	6	5	1	2	5		1	1			1												84	10.5	10.0
[illegible]	15 15				1		1	4	1	1	3	1	1		1								1									100	9.6	9.0
[illegible]	16 16					3	1	5	3	1	1	1																				100	7.3	7.0
[illegible]	16 16		1			1	7	4	3																							100	6.3	6.0
MIXED GAMMA-NEUTRON RADIATIONS																																		
0	0 32																															0		
[illegible]	0 20																															0		
200	5 32										2					1				1												16	13.4	13.[illegible]
[illegible]	8 20		1		1		1	1													2		2									40	13.9	13.5
[illegible]	14 20			1				3	1	2		1			1				3						1		1					70	12.8	10.0
391	10 32						1	2			1	1		1	1		1	1										1				31	12.0	13.0
[illegible]	17 20					2	2	1	5		5		1	2													1					85	9.6	8.0
429	17 32			1	1		1	1		2	2	3		1		2			2									1				53	11.6	11.0
[illegible]	20 20		1		3	1	8	2	3			1	1	2				1	1													100	7.7	6.5
531	30 32					1	2	2		2	1	9	3	8	1											1						94	11.2	11.0
[illegible]	19 19				1	3	4	2	1	1	4	2	1																			100	7.8	7.0
639	32 32				1		2	12	3	4	6	2	1	2																		100	6.5	8.0

*BOLD TYPE DENOTES THE INITIAL EXPOSURES AND ITALIC THE [illegible]OND EXPOSURE SERIES
**FIGURES IN POSTIRRADIATION DAY OF DEATH COLUMNS INDICATE NUMBER OF DEATHS ON THAT DAY

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* Ralston Purina Co., St. Louis, Missouri

per group) and were similarly irradiated (Table I). Exposures were extended into the supralethal dose range to permit a comparison with results obtained by exposing rats to comparable doses.

At the time of irradiation, the ages of the chinchillas ranged from 24 weeks to approximately 8 years* and their weights varied from 415 to 644 grams. All exposures were unilateral and employed minimal scatter conditions. Plexiglas† boxes (3-7/8" wide x 8-7/8" long x 5" high), constructed from sheets 1/8" thick, were used to confine the chinchillas during exposure. Depth dose measurements were made in chinchilla phantoms (Figure 1) using miniature tissue equivalent (T. E.) ionization chambers.[6] The phantoms were fabricated from Plexiglas tubing (3-1/4" O. D. - 2-7/8" I. D.) and filled with tissue equivalent fluid.[17] The phantoms were representative of

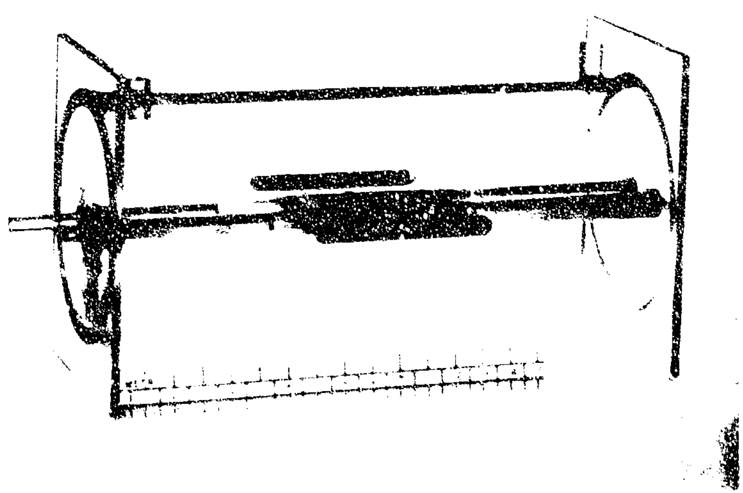

Figure 1. Chinchilla phantom showing placement of miniature tissue equivalent ionization chambers

* The precise ages of the chinchillas were unknown. The reported age range is based on the available growth curves[19] and estimated longevity[9] of these rodents.

† Acrylic plastic. Rohm and Haas Co., Philadelphia, Pennsylvania

the average weight and size of the chir hillas in this study. Two longitudinal grooves, diametrically opposing each other, were centered on the surface of the phantom. Each groove held three miniature T. E. chambers used to measure entrance or exit doses. Depth dose measurements at 1/4, 1/2, and 3/4 the diameter of the phantom were made with T.E. chambers positioned in Plexiglas tubes (5/16" O.D. - 3/16" I.D.) traversing the length of the cylinder. All depth dose measurements in the phantoms were made using the same exposure conditions as for the animal irradiations.

The results of depth dose measurements made in the chinchilla phantoms are shown in Figure 2. Categorizing the irradiations according to degree of uniformity of absorbed dose within the volume of interest,[11] both the x-ray and mixed gamma-eutron irradiations were Class B nonuniform exposures.

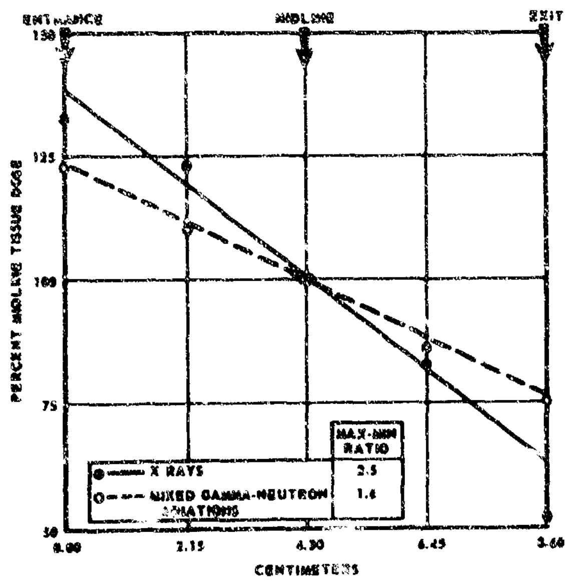

Figure 2. Dose profiles in unilaterally exposed chinchilla phantoms

The x-ray exposures were accomplished with the 360° radial beam from a 250 kVp x-ray generator operated at 30 mA. The inherent (1.2 mm beryllium) and added (0.95 mm copper) filtration resulted in a half value layer (HVL) of 1.9 mm of copper. Exposure boxes were placed in the radiation field so that the midline of each animal was 1 meter from the x-ray target (Figure 3).

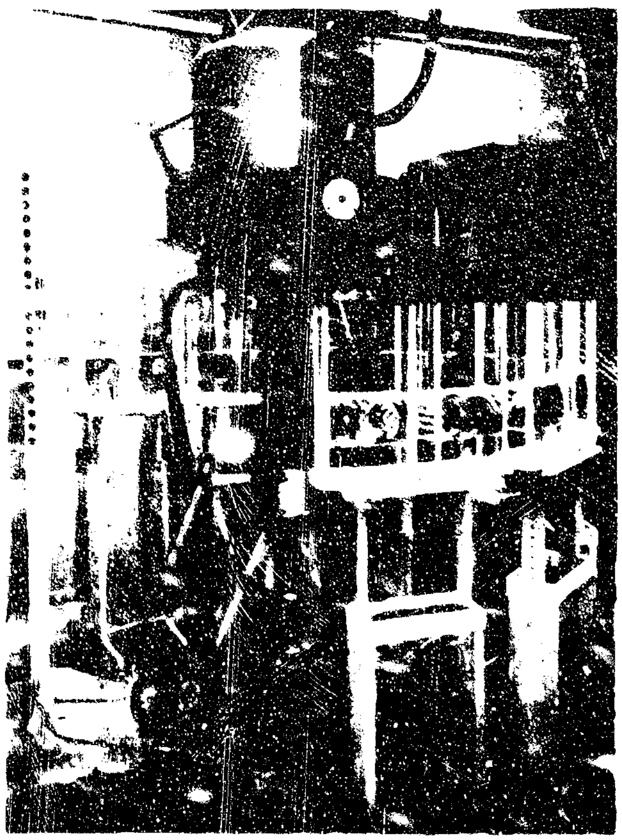

Figure 3. X-ray exposure array for chinchillas

The absorbed dose at the center of the animal was calculated from three factors. First, a Victoreen Roentgen Chamber was used to determine the exposure, free-in-air, at the position to be occupied by the center line of the animal. Positions were selected so that the variation in this quantity was less than 4 percent from the mean. Second, the ratio of the dose at the midline of a phantom (simulating the chinchilla in the irradiation position) to the exposure free-in-air, was obtained using miniature ionization chambers. Third, the conversion factor ($\bar{f}$) of 0.95 for muscle and for this quality of radiation was obtained from the ICRU Report 10b.[10] The product of these three factors gave the absorbed dose at the center line of the animal. The dose rate in all exposures was approximately 21 rads/min. Dose rate was monitored continuously during each exposure with a Victoreen rate meter in order to detect any changes in the output of the x-ray unit.

Figure 4 illustrates the array used for the exposures to mixed gamma-neutron radiations. The exposure boxes were positioned so that the midline of each animal was on an arc 292 cm from the vertical center line of the reactor core. This arc, located approximately in the middle of the exposure room, was in an exposure field in which the tissue kerma, free-in-air, did not vary by more than 4 percent from the mean.

Figure 4. Reactor exposure array for chinchillas

The absorbed dose at the midline of the animal was calculated from two factors. First, the tissue kerma, free-in-air, was calculated from measurements with a 50 cm^3 cavity tissue equivalent plastic* walled ionization chamber. Second, the ratio of the absorbed dose in the center of the phantom (simulating the chinchilla in the irradiation position) to the tissue kerma, free-in-air, was obtained. The product of these two quantities gave the absorbed dose at the center line of the animal. The absorbed dose rate for all exposures was approximately 19 rads/min.

Approximately 60 percent of the tissue kerma, free-in-air, is attributed to gamma rays, 30 percent to neutrons of energies greater than 10 keV and 10 percent to neutrons of lower energies. The effective energy† of the gamma component was between 1 and 2 MeV. Details of the reactor characteristics and methods of dosimetry used in this mixed radiation field have been previously described.[8,20]

Chinchilla deaths were recorded daily for 30 days following irradiation. The $LD_{50/30}$ values were calculated by subjecting the resulting data to probit analysis using a maximum likelihood method programmed for a digital computer. The resultant regression lines from the initial and second series of exposures were tested for homogeneity and parallelism, and the $LD_{50/30}$ values were tested for differences. The mean survival time for the decedents of each group was plotted and a "least squares" method employed to determine the best fitting lines. The 95 percent

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* Plastic supplied by Dr. F. R. Shonka, St. Procopius College, Lisle, Illinois. (Composition by weight: 76.1 percent carbon, 10.1 percent hydrogen, 5.2 percent oxygen, 3.5 percent nitrogen, 1.0 percent silicon, 2.0 percent calcium and 2.0 percent fluorine.)

† Depth dose studies, using Plexiglas rat phantoms, indicate that the deposition of energy by the gamma component of the reactor radiations was similar to that of ^{60}Co gamma rays.

confidence band for each regression line as a whole was computed using the method described by Natrella.[15]

No significant differences were found when the dose response regression lines and $LD_{50/30}$ values from the initial exposures were tested against their counterparts from the second series of exposures, nor was any significant difference found between the radiosensitivity of males and females. Since the results of the initial and the second series of exposures were similar, the data were combined and analyzed to simplify presentation.

III. RESULTS

Table I on page 2 summarizes the mortality data for the chinchillas. The raw data used for probit analysis and the resultant dose-response regression lines with their 95 percent confidence bands are displayed graphically in Figure 5.

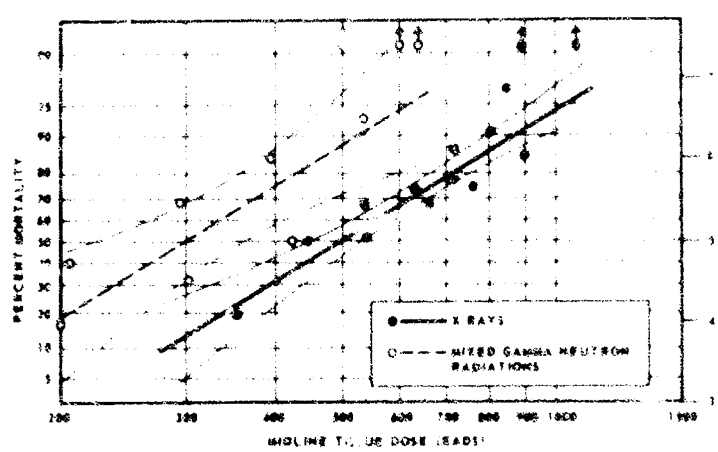

Figure 5. Dose-response regression lines and associated 95 percent confidence bands for the chinchilla as calculated by probit analysis. Plotted points represent raw data and arrows indicate the doses associated with 100 percent mortality.

The results of probit analysis and the calculated relative biological effectiveness (RBE) for the mixed gamma-neutron radiations are shown in Table II. The mean survival time regression lines and their 95 percent confidence bands for chinchillas exposed to x rays and mixed gamma-neutron radiations are shown in Figure 6.

Table II. Probit Analysis of Chinchilla 30-Day Mortality Data

Radiation source	Calculated lethal dose values*					Slope of regression line	RBE† for $LD_{50/30}$'s
	LD_{10}	LD_{30}	LD_{50}	LD_{70}	LD_{90}		
x ray	280 (209-335)‡	390 (323-438)	490 (435-531)	616 (574-658)	858 (789-971)	5.3	-
mixed gamma-neutron radiations	168 (69-227)	234 (137-292)	295 (212-360)	370 (299-487)	515 (413-899)	5.3	1.7

* Midline tissue dose (rads)

† 250 kVp x rays used as standard reference source

‡ 95 percent confidence limits shown in parentheses

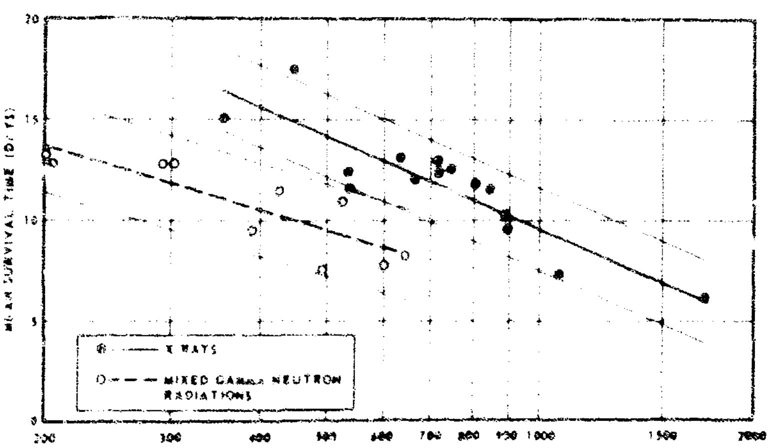

Figure 6. Survival time regression lines and associated 95 percent confidence limits for the chinchilla. The plotted points represent the mean values for each dose group listed in Table I.

In Table III, the mortality response of chinchillas exposed to supralethal doses of x rays is compared with the respective mortality response previously obtained in rats. A similar comparison after exposure to mixed gamma-neutron radiations is shown in Table IV.

Table III. Dose-Response Data of Rats and Chinchillas Exposed to Supralethal Doses of x rays

Midline Tissue Dose (rads)	Number of Animals	Postirradiation Day of Death (number of deaths on day indicated; days 5, 10, 15, 20, 25)	30-Day Percent Mortality	Median Survival Time (days)
Rat				
2052	48	7, 41	100.0	4.0
1231	48	44, 4	100.0	4.0
1026	48	21, 13, 5, 4, 1, 2, [illegible], 1	100.0	5.0
Chinchilla				
1786	16	1, 1, 7, 4, [illegible]	100.0	6.0
1072	16	2, 3, 5, 3, 1, 1, 1	100.0	7.0
893	47	1, 1, 2, 6, 4, 7, 6, 2, 3, 5, 1, 1, 1, 1, 1	89.4	9.5

Table IV. Dose-Response Data of Rats and Chinchillas Exposed to Supralethal Doses of Mixed Gamma-Neutron Radiations

Midline Tissue Dose (rads)	Number of Animals	Postirradiation Day of Death (number of deaths on day indicated; days 5, 10, 15, 20, 25)	30-Day Percent Mortality	Median Survival Time (days)
Rat				
734	48	24, 24	100.0	4.5
649	48	16, 30, 2	100.0	5.0
548	48	2, 3, 13, 9, 3, 4, 4, 3, 1, 1	100.0	7.0
Chinchilla				
639	32	1, 2, 12, 2, 4, 6, 2, 1, 2	100.0	8.0
601	19	1, 3, 4, 2, 1, 1, 4, 2, 1	100.0	7.0
531	32	1, 2, 2, 2, 1, 9, 3, 8, 1, 1	93.8	11.0

IV. DISCUSSION

Dose-mortality and survival-time values for the chinchilla indicate that the response of this rodent to doses of ionizing radiations in the lethal range is similar to that of mice and rats.[22] The lethal dose range for the chinchillas used in this study was much wider than for mice and rats. The increased width of the lethal range indicates that the chinchillas represented a heterogeneous population. This heterogeneity is reflected in the slopes of the probit regression lines (Figure 5) and the width of the associated 95 percent confidence bands (Table II). The slopes of the probit regression lines are less than half of the values reported for rats[22] and mice.[2,22] It has been shown that the radiosensitivity of mice changes with age.[1] Assuming that this is also true for other rodents, the wide range of ages of the chinchillas in this study could account for much of the heterogeneity that was observed.

The 16 early deaths (prior to the 5th postirradiation day) observed in chinchillas exposed to x rays or to mixed gamma-neutron radiations (Table I) in the lethal dose range apparently resulted from a natural characteristic of the species, rather than an effect of radiation. Chinchillas are extremely sensitive to some forms of stress. An animal may appear quite normal and within minutes die from shock induced by conditions ordinarily not considered lethally stressful. The gross pathology seen at the necropsy of animals dying early was similar to that described by T-W-Fiennes[23] for chinchillas which died from shock and was not characteristic of radiation injury. These early deaths were included in the probit analysis but had no significant effect on the mortality values calculated in this study.

A unique characteristic of the chinchilla found in this study is its apparent radioresistance to the gastrointestinal modality of death when compared to the Sprague-Dawley rat. For example, it has been our repeated observation that Sprague-Dawley rats are, within the 500- to 700-rad dose range of mixed gamma-neutron radiations (Table IV), quite susceptible to the gastrointestinal modality of death. (Other investigators have made similar observations in mice following exposure to neutron or modified fission spectrum radiation doses of less than 500 rads.[5,24]) However, the chinchilla did not demonstrate this susceptibility when subjected to the same mixed gamma-neutron radiations and comparable dose range. The resistance of the chinchilla to the gastrointestinal mode of death was tested further by irradiating animals at doses as high as 1786 rads of 250 kVp x rays (Table III). The mortality results indicated that the supralethal doses of x rays used did not shift the chinchilla deaths into the gastrointestinal temporal range. The mortality response of the chinchilla in the supralethal dose range does not agree with the results reported for mice and rats wherein classical gastrointestinal deaths are found in small rodents exposed to 1000 R or more of gamma or x rays.[4,13,16,18]

Mean survival time for the decedents of each exposed group was plotted against dose and regression lines fitted to the data points for each radiation type (Figure 6). For those portions of the regression lines where equal doses can be compared, the chinchillas exposed to radiations from the reactor have significantly shorter survival times than those exposed to x rays. Similar results were reported for mice[3,5,22,24] and rats.[22]

V. SUMMARY

Chinchillas were unilaterally exposed to whole body doses of mixed gamma-neutron (reactor) radiations or 250 kVp x rays. Dose rates were approximately 20 rads/min. Midline tissue doses from 200 to 639 rads of mixed gamma-neutron radiations and from 357 to 1786 rads of x rays were used. The $LD_{50/30}$ values were calculated to be 29[illegible] rads for the mixed gamma-neutron radiations and 490 rads for the x rays. Using the $LD_{50/30}$ value as the end point, the relative biological effectiveness (RBE) of the reactor radiations was 1.7. The heterogeneity in age of the chinchillas was interpreted as being responsible for the rather wide variations in mortality response. At comparable supralethal doses, chinchilla survival times were not as markedly reduced as the rat survival times.

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13. ABSTRACT

The chinchilla's acute mortality response to mixed gamma-neutron radiations of the AFRRI-TRIGA reactor and to 250 kVp x rays was studied. Unilateral whole body irradiations were accomplished at doses from 200 to 639 rads of mixed gamma-neutron radiations and from 357 to 1786 rads of x rays. All radiations were delivered at approximately 20 rads/min, and doses are reported as midline tissue doses. The $LD_{50/30}$ values calculated for the mixed gamma-neutron radiations and for the x rays were 295 and 490 rads, respectively. Using 30-day median lethality as the end point, the RBE of the mixed gamma-neutron radiations was 1.7. The wide lethal dose range obtained was attributed to a high degree of variation in age of the chinchillas. In contrast to previous experience in rodents, the chinchilla showed a relative resistance to the classically described gastrointestinal modality of radiation death.

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