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Pacific Northwest Research Foundation
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Seattle, Washington 98104

Terminal
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Division of Biomedical and Environmental Research
Energy Developement and Research Administration
Washington, D.C. 20545

Title

EFFECTS OF IONIZING RADIATION ON THE TESTICULAR FUNCTION OF MAN

CONTRACT # AT (45-1) 1780

Principal Investigator: Mavis J. Rowley

Contract ending July 31, 1974

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INTRODUCTION AND PROJECT HISTORY

In 1963, a project was initiated by Carl G. Heller, M.D., Ph.D. of the Division of Reproductive Physiology of the Pacific Northwest Research Foundation, to determine the effects of graded doses of ionizing radiation (in this case, X-ray irradiation) upon the human male reproductive system. Actual testicular irradiations were begun in August of that year, using inmate volunteers from the Oregon State Penitentiary. During the years irradiations were performed (between August 1963 and May 1971), a total of 67 men received acute testicular irradiation at dosages of 8 rads to 600 rads (see TABLE I).

During 1963, 1965 and 1967 ad hoc advisory/review committees were assembled by the Atomic Energy Commission to give overview and direction to this program. As a result, the parameters measured in early years were changed as more sophisticated methods became available, and extensive statistical analysis was performed.

The basic objectives of this research program were 1) to determine the exact nature of the cytological defect produced in the development of the germinal epithelium and to relate the extent of the defect to dosage and times; 2) to find the minimal dosage that will affect the germinal epithelium; 3) to determine the time of recovery from any given dosage; 4) to determine the minimal dosage that leads to permanent damage of spermatogenic cells; 5) to determine the simultaneous effects of any dosage upon Leydig cell cytology; 6) to determine the influence of any given radiation-produced testicular

TABLE I TABULATION OF EACH X-RAY DOSE ADMINISTERED, THE AGES OF THE SUBJECTS AT THE TIME AND THE DOSE GROUP EACH IRRADIATION WAS PLACED INTO FOR DATA ANALYSIS.

Dose Grouping for Data Analysis	Actual X-ray Dose (rads)	Irradiation Date	Subject Age (years)
8r	8.0	10-12-67	28
	8.0[1]	10-12-67	27
	8.0	10-12-67	29
	8.0[4]	10-12-67	27
10r	9.4	4-14-65	41
	9.7	2- 2-67	43
	10.5	2-17-67	36
15r	13.9	11-23-63	30
20r	18.3	9-29-65	25
	18.8	8- 5-64	26
	18.8	8- 5-64	--
	18.8	4-14-65	31
	18.9	10-13-65	36
	20.0	5- 6-71	--
	20.0	9-12-70	42
	20.0	5- 6-71	27
25r	23.1[2]	11-23-63	28
	23.6[2]	10-13-65	30
50r	45.8	9-29-65	31
	45.8[3]	9-29-65	42
	46.3[3]	11-23-63	40
	47.0	9-14-63	32
	47.0	9-14-63	52

75r	75.0	5- 6-71	28
	75.0	1- 9-71	37
	77.0	10-12-67	30
	77.0	10-12-67	43
	77.0	10-12-67	34
	77.0	10-12-67	40
	78.0	9-12-70	--
100r	92.6	11-23-63	31
	93.1	12-10-64	24
	93.5	8-22-63	30
	93.5	8-22-63	--
	93.6	3-19-64	31
	93.6	3-19-64	37
	94.0	9-14-63	--
	97.5	2- 2-67	46
	98.2	10-13-65	38
	101.0	2-17-66	28
200r	183.3	9-29-65	39
	188.1	4-14-65	27
	188.7	8-17-63	34
	188.7	8-17-63	--
	191.5	1- 5-67	43
	191.5	1- 5-67	37
	200.0	5- 6-71	30
	200.0	9-12-70	29
	200.0	9-12-70	36
	200.0	9-12-70	--
	200.0	5- 6-71	26
	200.0	1- 9-71	36

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230r	228.0	10-12-67	41
300r	280.5	8-22-63	37
	280.5[5]	8-22-63	29
400r	367.0	9-14-63	40
	375.3	8- 5-64	25
600r	547.5	7-21-64	49
	549.9	2-18-65	41
	554.4	3-14-65	44
	556.4	8- 5-64	28
	564.5	4-14-65	--
	600.0	11-14-68	35
	600.0	3-21-70	41
	600.0[1]	11-14-68	28
	600.0[6]	3-21-70	49
	600.0	11-14-68	30
	600.0	1- 9-71	34
	600.0	1- 9-71	25
	600.0[4]	5- 6-71	31
	600.0	11-14-68	33
	640.0	9-12-70	37
50r TOTAL	Received 11 doses of 4.6r (av.)	From 2-18-65 to 4-29-65 Each dose separated by 7 days	34

Superscript numbers 1 through 6 refer to the six subjects who received more than one X-ray dose, e.g. both 25r doses were received by the same subject.

alteration upon other parameters such as the excretion of total gonadotropic hormones, follicle-stimulating hormone (FSH), luteinizing hormone (LH) (also known as interstitial cell-stimulating hormone or ICSH), estrogens and androgenic hormones; 7) to determine the influence of any given radiation-produced testicular alteration upon plasma FSH, LH and testosterone; and 8) complete statistical examination of all parameters and their interactions.

Unfortunately, although the radiation of subjects was completed, the projected follow-up of irradiated volunteers was cut short in February of 1973 by the decision of the State of Oregon Director of Correctional Institutions to discontinue research following Dr. Heller's disability. Because of Dr. Heller's incapacity the Principal Investigator was changed to Mavis J. Rowley (formally approved on February 22, 1973) and terminal year support granted through July 31, 1974 to implement the closing of the laboratory at the Pacific Northwest Research Foundation.

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NUMBER OF PLASMA SAMPLES AVAILABLE FOR PLASMA TESTOSTERONE DETERMINATIONS BEFORE AND AFTER TESTICULAR X-RAY IRRADIATION

Dose	Subject #	Control or pre-irradiation samples available	Post-irradiation samples available	Range of time after irradiation for which samples are available
600r	DELETED	0	33	2 months to 4 years
	DELETED	2	16	3 weeks to 1 year 5 months
	DELETED	0	6	1 week to 2½ years
	DELETED	5	28	1 week to 2½ years
	DELETED	1	32	1 week to 3½ years
	DELETED	0	1	about 5 years
	DELETED	3	12	1 month to 1½ years
	DELETED	0	4	about 5 years
	DELETED	3	8	7 months to 1½ years
	DELETED	1	15	2 weeks to 2 years
	DELETED	1	33	1 week to 3 years
200r	DELETED	6	15	1 day to 1 year
	DELETED	3	17	2 months to 4 years 2 months
	DELETED	1	9	5 months to 1 year 2 months
	DELETED	0	2	about 1 year
	DELETED	4	12	3 months to 1 year 3 months
	DELETED	4	5	3 months to 6 months
	DELETED	0	11	13 months to 4 years
	DELETED	0	17	1 day to 1 year 2 months
	DELETED	6	6	2 weeks to 8 months
	DELETED	0	6	about 6 years
78r	DELETED	6	23	1 day to 1 year 3 months
	DELETED	0	10	about 4 years
	DELETED	3	16	3 months to 2 years
	DELETED	6	7	2 weeks to 5 months
	DELETED	3	4	4 months to 7 months
20r	DELETED	6	7	1 day to 4 months
	DELETED	13	14	1 day to 7 months
	DELETED	6	7	3 months to 1 year
10r	DELETED	2	3	3 months to 4 months
8r	DELETED	0	6	about 4 years

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MATERIALS AND METHODS USED FOR DATA COLLECTION

Selection of Subjects

The 67 subjects for this study were healthy normal men (see APPENDIX A) between the ages of 25 and 52 years. Their reproductive normalcy was determined prior to irradiation by physical examination, examination of testicular biopsy specimens, sperm concentration and sperm morphology. Levels of urinary total gonadotropins, luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, estrogen and/or plasma LH, FSH, and testosterone were also determined. Additional routine tests such as blood cytology, urinalysis and various blood chemistry tests were evaluated during the pre- and post-irradiation period.

A Device to Deliver Uniform X-Ray Doses

Equipment was required to deliver a uniform, accurately predetermined dose of X-radiation throughout both testes of human volunteers. Building modifications were undesirable, therefore the apparatus was to be portable, operable from 115V power supply, independent of plumbing and with built-in radiological protection. Exposure doses, uniform to within $\pm$ 5% of the stated average were to be delivered at the rate of approximately 100 roentgens per minute.

The equipment provided (FIGURES 1 & 2) consisted of two air-cooled industrial X-ray units, with tungsten targets, operating at approximately 140 KVP with up to a 5 m.a. tube current, having an inherent filtration of 0.14" glass plus 0.12" mg, equivalent to 2 mm. Al.

PLAN OF IRRADIATION UNIT

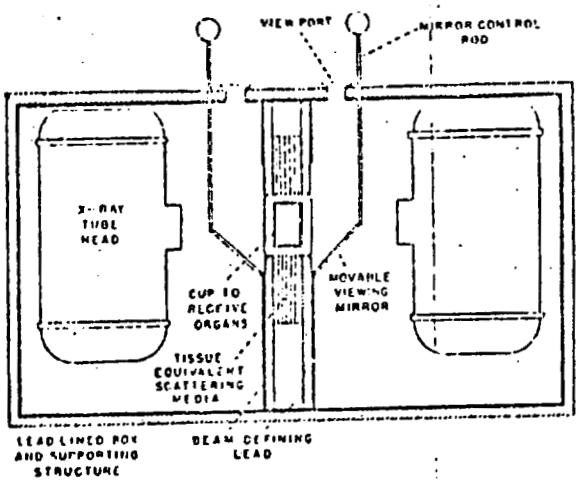

FIGURE 1

SECTION THROUGH IRRADIATION UNIT

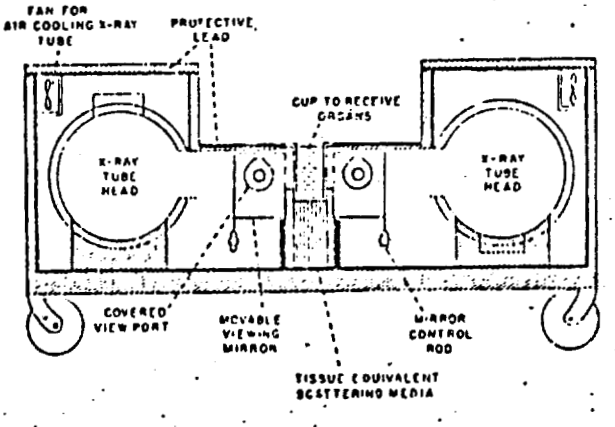

FIGURE 2

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These units were mounted in a lead-lined box with their beams horizontally opposed on a common axis. The X-ray beams were incident on a block of tissue equivalent material 20 cms x 20 cms x 6 cms thick, placed mid-way between the tubes. Inserted into this block of material in the middle of the upper edge was a transparent 10 cms x 15 cms x 6 cms vessel of .125" plexiglass.

In use, the plexiglass vessel was filled with warm (93 - 94°F) water which served two purposes, a) physiological, to obtain descent of the testes into the vessel, and b) physical, making the testes, water vessel and surrounding material one tissue equivalent mass, thereby making the dose distributions in the testes independent of the natural variations in the geometry of the subject.

In application, the cup was filled with water at scrotal temperature and the subject laid prone with testicles suspended in the water. The level of the testicles in the cup was viewed in the mirrors through the view ports. When the testicles were below the 1.7 cm level, the mirrors were swung out of the X-ray beam path and the predetermined dose delivered. The unit was recalibrated at the termination of the experiment. Our records show less than 1% change in output during a run.

X-Ray Calibration Check

One independent check of the X-ray calibrations was made using R-meters by a physicist at Battelle Northwest Laboratories. He confirmed that " Our independent calibrations agreed with those of

the experimenters within the accuracy expected from the instruments used ..... The standard deviation between our results was about 3%. " A second independent check was made using a biological standard, by Dr. Eugene F. Oakberg of Oak Ridge National Laboratory. A special mouse-holding chamber that would fit into the water box of the X-ray machine was constructed. Dr. Oakberg's genetically pure strain of mice was used. Mice were irradiated at various dosage levels, then autopsied at 72 hours after X-ray. The preserved testicular specimens were analyzed and the results compared to results obtained using equipment at other institutions (FIGURE 3).

Data on Irradiation of Subjects

Sixty-seven subjects received acute testicular irradiation in doses between 8r and 600r. Six subjects were irradiated a second time, and one subject a third time, following complete recovery from the initial dosage. Of these, three received the identical dosage on each of the two occasions, three received two different dosages, and one received three different dosages. One subject was given weekly irradiations of 5r per week for eleven weeks. Each dosage given is listed separately in TABLE I.

Post-Irradiation Evaluations

For each parameter studied each subject served as his own control. Post-irradiation observations were made on each parameter mentioned above. Serial testicular biopsies were taken following 42 irradiations. After 13 irradiations biopsies were not taken during the post-irradiation period in order to specifically evaluate sperm

X-RAY IRRADIATION

SUBJECT NUMBER	DOSE (RADS)	DATE ADMINISTERED	BIOPSIES: Control		First 90 days following X-ray		After 90 days following X-ray		DETERMINATIONS: PLASMA			URINE			SEMEN EVALUATIONS	
			Yes	No	Yes	No	Yes	No	T	LH	FSH	T	LH	FSH	Yes	No
DELETED	600	11-14-68	X		X		X		X	X	X	X	X	X	X	
DELETED	200	6-17-63	X			X	X			X	X	X	X	X	X	
DELETED	100	11-23-63	X			X	X						X		X	
DELETED	400	9-14-63	X		X		X						X		X	
DELETED	15	11-23-63	X			X	X						X		X	
DELETED	100	12-10-64	X			X		X					X		X	
DELETED	8	10-12-67	X			X		X		X			X	X	X	
DELETED	600	11-14-68	X		X		X			X			X	X	X	
DELETED	600	7-21-64	X			X	X			X	X	X	X	X	X	
DELETED	20	8-5-64	X		X		X								X	
DELETED	100	3-19-64	X		X		X					X			X	
DELETED	600	8-5-64	X		X		X			X	X	X	X	X	X	
DELETED	300	8-22-63	X		X		X					X	X	X	X	
DELETED	200	9-12-70	X		X		X			X	X					
DELETED	200	6-17-63	X		X		X						X			X
DELETED	100	9-14-63	X		X			X					X			X
DELETED	600	4-14-65	X		X		X					X	X		X	
DELETED	8	10-12-67	X			X	X									

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X-RAY IRRADIATION

SUBJECT NUMBER	DOSE (RADS)	DATE ADMINISTERED	BIOPSIES: Control Yes	BIOPSIES: Control No	BIOPSIES: First 90 days following X-ray Yes	BIOPSIES: First 90 days following X-ray No	BIOPSIES: After 90 days following X-ray Yes	BIOPSIES: After 90 days following X-ray No	DETERMINATIONS: PLASMA T	PLASMA LH	PLASMA FSH	URINE T	URINE LH	URINE FSH	SEMEN EVALUATION Yes	SEMEN EVALUATION No
DELETED	100	3-19-64	X		X			X							X	
DELETED	100	10-13-65	X		X		X					X			X	
DELETED	400	8-5-64	X		X		X					X		X	X	
DELETED	5	2-18-65	X			X	X								X	
DELETED	5	2-25-65	X			X	X					X			X	
DELETED	5	3-4-65	X			X	X					X			X	
DELETED	5	3-10-65	X			X	X					X	X		X	
DELETED	5	3-18-65	X			X	X					X			X	
DELETED	5	3-25-65	X			X	X					X			X	
DELETED	5	4-1-65	X			X	X					X			X	
DELETED	5	4-8-65	X			X	X					X			X	
DELETED	5	4-15-65	X			X	X					X			X	
DELETED	5	4-22-65	X			X	X					X			X	
DELETED	5	4-29-65	X			X	X					X			X	
DELETED	20	8-5-64	X			X	X					X			X	
DELETED	10	4-14-65	X		X		X					X			X	
DELETED	20	4-14-65	X		X		X					X			X	
DELETED	600	3-4-65	X			X	X									

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X-RAY IRRADIATION

SUBJECT NUMBER	DOSE (RADS)	DATE ADMINISTERED	BIOPSIES: Control Yes	BIOPSIES: Control No	BIOPSIES: First 90 days following X-ray Yes	BIOPSIES: First 90 days following X-ray No	BIOPSIES: After 90 days following X-ray Yes	BIOPSIES: After 90 days following X-ray No	DETERMINATIONS: PLASMA T	DETERMINATIONS: PLASMA LH	DETERMINATIONS: PLASMA FSH	DETERMINATIONS: URINE T	DETERMINATIONS: URINE LH	DETERMINATIONS: URINE FSH	SEMEN EVALUATION Yes	SEMEN EVALUATION No
DELETED	200	4-14-65	X		X		X					X		X	X	
DELETED	20	9-29-65	X			X	X					X		X	X	
DELETED	100	2-17-66	X		X		X					X		X	X	
DELETED	50	9-29-65	X			X	X					X		X	X	
DELETED	200	2-17-66	X			X	X					X	X	X	X	
DELETED	10	2-17-66	X		X			X				X			X	
DELETED	200	9-29-65	X			X	X		(SEE HCG)			X		X	X	
DELETED	10	2-2-67	X		X		X			X		X	X	X		X
DELETED	77	10-12-67	X			X		X				X	X	X	X	
DELETED	8	10-12-67	X			X	X			X		X	X	X	X	
DELETED	600	5-6-71	X		X		X		X	X	X					
DELETED	77	10-12-67	X			X	X					X	X	X	X	
DELETED	8	10-12-67	X			X	X						X	X	X	
DELETED	100	2-2-67	X		X		X			X	X	X		X		X
DELETED	600	3-21-70	X		X		X			X	X			X		X
DELETED	77	10-12-67	X			X	X					X	X	X	X	
DELETED	77	10-12-67	X			X	X			X		X	X	X	X	
DELETED	[illegible]	1-[illegible]-67				X	X									

X-RAY IRRADIATION

SUBJECT NUMBER	DOSE (RADS)	DATE ADMINISTERED	BIOPSIES: Control Yes	BIOPSIES: Control No	BIOPSIES: First 90 days following X-ray Yes	BIOPSIES: First 90 days following X-ray No	BIOPSIES: After 90 days following X-ray Yes	BIOPSIES: After 90 days following X-ray No	DETERMINATIONS: PLASMA T	PLASMA LH	PLASMA FSH	URINE T	URINE LH	URINE FSH	SEMEN EVALUATION Yes	SEMEN EVALUATION No
DELETED	20	5-6-71	X		X		X			X	X				X	
DELETED	600	11-14-68	X		X		X			X	X	X	X	X	X	
DELETED	600	11-14-68	X		X		X			X	X	X	X	X	X	
DELETED	20	9-12-70	X		X		X			X	X				X	
DELETED	600	3-21-70	X		X		X			X	X			X		X
DELETED	600	9-12-70	X			X	X		X	X	X				X	
DELETED	200	9-12-70	X		X		X			X	X					X
DELETED	78	9-12-70	X			X	X			X	X				X	
DELETED	200	9-12-70	X		X		X		X	X	X					X
DELETED	600	1-9-71	X		X		X		X	X	X				X	
DELETED	600	1-9-71	X		X		X		X	X	X				X	
DELETED	75	1-9-71	X		X		X			X	X				X	
DELETED	200	1-9-71	X		X		X			X	X				X	
DELETED	78	5-6-71	X		X		X			X	X				X	
DELETED	200	5-6-71	X		X		X		X	X	X				X	
DELETED	200	5-6-71	X			X	X			X	X				X	
DELETED	20	5-6-71	X			X	X			X					X	
DELETED	235	10-12-67	X			X	X			X	X	X	X	X		

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X-RAY IRRADIATION

SUBJECT NUMBER	DOSE (RADS)	DATE ADMINISTERED	BIOPSIES: Control		BIOPSIES: First 90 days following X-ray		BIOPSIES: After 90 days following X-ray		DETERMINATIONS: PLASMA			DETERMINATIONS: URINE			SEMEN EVALUATIONS	
			Yes	No	Yes	No	Yes	No	T	LH	FSH	T	LH	FSH	Yes	No
DELETED	100	8-22-63		X		X	X			X	X	X	X	X	X	
DELETED	100	8-22-63		X	X		X						X			X
DELETED	50	9-14-63	X			X	X					X	X	X	X	
DELETED	50	9-14-63		X	X		X					X	X			X
DELETED	25	11-23-63	X		X		X					X	X	X		X
DELETED	25	10-13-65	X		X		X					X		X		X
DELETED	50	11-23-63	X			X		X					X		X	
DELETED	50	9-29-65	X			X	X					X			X	
DELETED	20	10-13-65	X		X		X					X				X

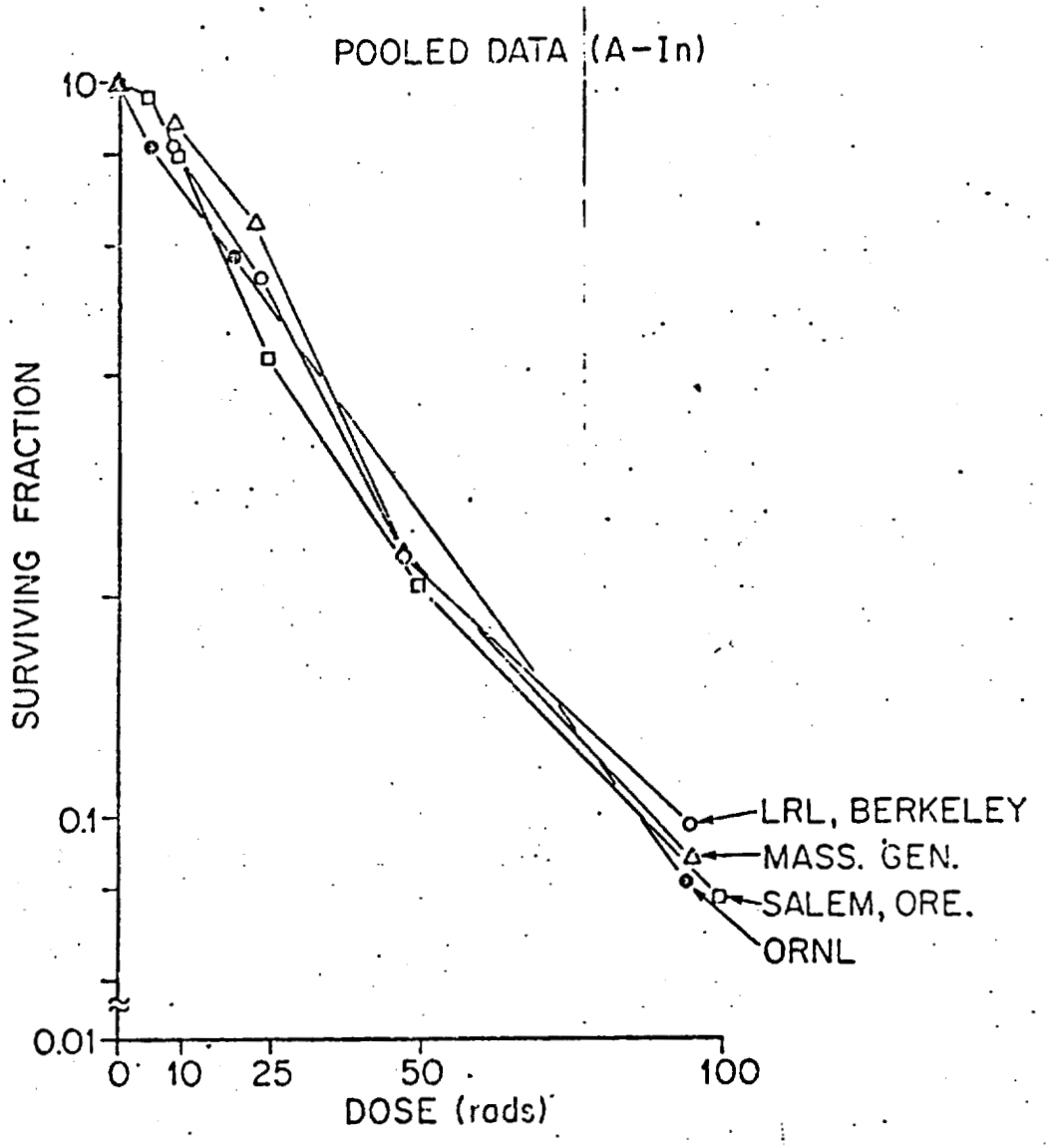
POOLED DATA (A-In)
10
0.1
0.01
SURVIVING FRACTION
0
10
25
50
100
DOSE (rads)
LRL, BERKELEY
MASS. GEN.
SALEM, ORE.
ORNL

FIGURE 3

and hormonal evaluations [6]. After 19 irradiations, biopsies were avoided only during the first 90 day cell depletion period [6] (subject receiving 5r per week tabulated as one irradiation only). All biopsies were prepared for light microscopy [7] and in the later years of the study for electron microscopy [8]. Germinal cell and Leydig cell quantitation methods were developed [3, 5] and utilized on material fixed for light microscopy.

Sperm counts were performed using the haemocytometer method on samples submitted weekly by each volunteer. Sperm morphology [9] was examined by Dr. John MacLeod of Cornell Medical School.

During the first years of this study, urinary steroid and hormone evaluations were used exclusively. As plasma methods became available, urinary evaluations were phased out. The urinary gonadotropins were concentrated using the speed-filter and kaolin adsorption method of Albert [10]. Total gonadotropins were bioassayed by the method of Lauson, Heller and Sevringhaus [11]. Luteinizing hormone bioassay was conducted using the male hypophysectomized rat method of Greep, et al. [12]. The Steelman-Pohley assay [13] was used for FSH. Estrogens were bioassayed using the method of Lauson, Heller, Golden and Sevringhaus [14]. Urinary testosterone and epitestosterone levels were determined using gas-liquid chromatography [15, 16].

Plasma testosterone was determined using the method of Murphy [17]. Radioimmunoassay of plasma FSH and LH was performed using modifications of methods by Odell, et al. [18, 19].

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REPOSITORY: DOE-RICHLAND
COLLECTION: GSS HUMAN TEST SUBJECTS STUDIES
PRISONER STUDY

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FOLDER: 1780 - HELLER PACIFIC NW RES. FOUNDA.

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