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THE STUDY OF IRRADIATION EFFECTS ON THE HUMAN TESTIS: INCLUDING HISTOLOGIC, CHROMOSOMAL AND HORMONAL ASPECTS

Terminal Report

January 31, 1973

C. Alvin Paulsen, M.D.
Professor of Medicine
University of Washington School of Medicine
Seattle, Washington

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Introduction

The final period of this research project involved primarily an extension of our long-term post-irradiation observations and an evaluation of concepts derived from the data collected during the nine-year period of this study.

Table I summarizes the final status of the 232 volunteers who were initially accepted for the program. Of these, 64 men were irradiated with acute doses of x-ray ranging from 7.5 to 400r (f factor = 0.95). Subsequent observations were then made for periods of up to 2225 days, or 6.1 years.

Fifty-three of the 64 men who were either irradiated, or who received a sham irradiation procedure, had a bilateral vasectomy prior to their termination of the study. A tabulation of the numbers used at each exposure level is given in Table I (A-1).

All volunteers signed agreements to be vasectomized at the end of the study if so requested. The reasons for not performing a vasectomy on the eleven men are outlined in Table I (A-2).

Table II summarizes the final status of the remaining volunteers at the termination of the project in March, 1972.

One of the important considerations in either whole-body irradiation, or x-rays directed toward the testes is the effect on spermatogenesis and Leydig cell function, which is the testosterone producing component of the testes. Therefore, seminal fluid analyses, testicular histology, urinary follicle-stimulating hormone (FSH), luteinizing hormone (LH) titers, and serum LH and testosterone levels were used to evaluate such changes that might be induced by graded doses of acute x-ray exposure.

As the program developed we focused our attention primarily on changes in sperm production, testicular histology, and FSH levels in the urine.

This hormone, in a manner that is not yet fully understood, is inversely correlated with spermatogenesis. This relationship is not linear, but is expressed only when spermatogenesis is damaged. It is not clear whether the elevated FSH levels are directly related to germ-cell damage, or whether the correlation is associated with alterations in the physiology of the Sertoli cell. One function of the Sertoli cells is to transport nutrients to the developing germ cells which are embedded within the Sertoli cell cytoplasm. See Enclosure I (Leonard, et al. J. Clin. Endocr. 34 (1): 209-214, 1972).

TABLE I. STATUS OF INMATE VOLUNTEERS AS OF MARCH, 1972

A. Total number of volunteers who were either irradiated or sham irradiated with acute x-ray doses to the mid-organ range of the testes:

1. Numbers of men per dose level:

400r	8	
100r	25	
50r	8	
30r	5	
15r	8	
7.5r	6	
Sham irradiation only	4	Total 64

2. Number of irradiated volunteers who did not have a vasectomy prior to termination of program:

a. Declined and released from prison	2	
Declined and still in prison	1	
Released earlier than scheduled vasectomy	1	
Mutual agreement (myocardial infarct and life sentence)	1	
Not required because of Sertoli-cell-only syndrome	1	
Vasectomy prior to prison sentence	1	
Not required for sham irradiation procedure	3	
Agreement with prison physician for psychiatric reasons	1	
	11	
b. Number of irradiated volunteers who had a bilateral vasectomy prior to release from program:	53	Total 64

B. Disposition of all inmate volunteers accepted for the project where at least control observations were obtained — Total 232

a. Number of men irradiated	64	
b. Released from program for personal, administrative or medical reasons	43	
c. Control groups, including studies such as effect of biopsy or of wearing a suspensory on sperm production; also those awaiting irradiation studies when the program was terminated	125	Total 232

TABLE II. SUMMARY OF FINAL IRRADIATION VOLUNTEERS' BIOPSY, VASECTOMY AND FSH STATUS

RV No.	X-ray Date	X-ray Dose	Final Biopsy & Vasectomy Date	Final FSH Levels as Compared to Control	Final Seminal Fluid Range	Time Interval After X-ray
26	6-24-64	400r	6-25-70	Normal	Control level	6 years
4	9-9-64	100r	10-14-70	Normal	Control level	6 years
32	5-3-64	100r	5-12-70	Normal	Control level	5 years
74	9-22-65	100r	10-14-70 (no vasectomy*)	Normal	Azoo-spermia	5 years
149	9-11-68	100r	12-9-70	Normal	Control level	2 years
192	12-11-68	100r	12-9-70	Normal	1/3 Control level	2 years
78	11-30-67	50r	5-12-70	Normal	Control level	2½ years
147	6-19-68	50r	10-14-70	Normal	Control level	2½ years
144	6-19-68	50r	10-14-70	Normal	Control level	2½ years
133	6-19-68	50r	5-12-70 biopsy, no vasectomy	Normal	Control level	2 years
31	2-19-65	15r	Released 12-9-70 No biopsy or vas.	Normal	Control level	6 years
141	6-19-68	7.5r	6-25-70	Normal	Control level	2 years

* Vasectomized prior to starting program

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Spermatogenesis

Enclosure 2 (Thorslund & Paulsen, The National Symposium on Natural and Manmade Radiation in Space, March 2, 1971, NASA Document No. 2440: 229-232, 1972) may be summarized as follows:

Direct cell kill and or inhibition of mitosis have been suggested as two possible mechanisms to explain the occurrence of absolute sterility following irradiation of the testes. In order to obtain information on the existence and dose dependency of the mechanisms for man, a controlled study was initiated in 1963. Normal adult inmate volunteers at the Washington State Pentitentiary in Walla Walla participated in the experiment. Sixty-four of the men received a single mid-organ dose to both of their testes, ranging from 7.5 to 400r (f=0.95). The remaining men served as various control groups. Testicular function was evaluated primarily on the basis of weekly seminal fluid examinations where the response, or end-point, was taken to be azoospermia or complete temporary sterility. In order to obtain direct information on testicular morphological changes periodic unilateral testicular biopsies were also performed on a number of individuals in both the exposed and non-exposed groups.

It was deduced from the resulting length of the pre-sterile period and sterile period data that both mechanisms, i.e., cell kill and "mitosis halting" were in operation. Estimates of ED_{50} were 75r for cell kill and 27r for "mitosis halting". The maximum observed sterile period was 529 days with eventual recovery observed in each individual where the follow-up time interval was adequate. Thus man appears to be highly radiosenstive with respect to temporary sterility, but quite radioresistant with respect to permanent sterility.

Table III summarizes the seminal fluid analysis data for the individuals in each x-ray dose group, compared with their mean control sperm concentrations. Column 5 shows a positive correlation between the dose and the time required to reach azoospermia, but this correlation is not pronounced except at the extreme lower and upper dose ranges.

The duration of azoospermia (column 7) appears to exhibit the best correlation with the dose administered. Furthermore the magnitude of the dose is closely related to the time required to re-enter the sperm count control range (column 8). It is noteworthy that all men who were followed for a sufficient time eventually regained their pre-irradiation sperm production level even at the 400r dose level.

Doses of 7.5 and 15r did not produce either temporary azoo- or oligospermia although a definite reduction in sperm production was seen in 3 men at the 15r exposure level. It should be noted that our criterion for oligospermia is a consistent reduction from <1.0 to 5 million sperm per cc; a reduction indicates a consistent and obvious decrease from the individual's control levels, and azoospermia is no detectable sperm, as determined by microscopically scanning a well-mixed drop of undiluted seminal fluid.

The 30r exposure produced azoospermia in two of four men, and temporary oligospermia occurred in the remaining two. At 50r temporary azoospermia occurred in 5 of 8 individuals, and in three oligospermia was noted. All subjects at the 100 and 400r exposure levels, for whom adequate follow-up data were available, were reduced to azoospermia lasting for varying periods of time.

TABLE III. EFFECT OF GRADED DOSES OF X-RAY, DELIVERED ACUTELY TO THE TESTES, ON HUMAN SPERM PRODUCTION (million per cc)

RV No.	Control Sperm Counts Mean	Control Sperm Counts Range	X-Ray Dose (f = 0.95)	Observed Days of Azoospermia First Day	Observed Days of Azoospermia Last Day	Observed Days of Azoospermia Duration	First Day to Reach Control Range (*)	Highest Post Irradiation Sperm Count	Total Days of Observation After X-ray
10	31	9 - 55	7.5r	No response				125	1280
119	126	84 - 175		No response				91	272
76	214	104 - 296		No response				377	482
99	216	103 - 379		No response				460	621
141	98	31 - 233		No response				172	736
20	211	95 - 358	15r	Significant reduction			360	221	1463
18	163	82 - 286		No response				424	306
31	220	80 - 416		No response				575	2118
41	324	199 - 652		Significant reduction			*	100	305
39	239	136 - 427		No response				246	201
163	82	42 - 157		Significant reduction			307	107	509
154	70	19 - 126		No response				74	175
72	169	78 - 274		No response				208	230
21	69	26 - 125	30r	Oligospermia			*	3	141
30	174	76 - 300		194	201	7	*	7	215
34	55	24 - 77		Oligospermia			*	9	263
29	19	4 - 51		95	277	182	524	36	928
101	272	175 - 617					*		13
17	60	12 - 186	50r	202	251	49	586	53	975
8	23	5 - 52		115	185	70	344	5	1391
78	131	79 - 261		Oligospermia			382	164	887
15	225	135 - 336		171	255	84	574	340	1189
147	64	24 - 124		160	251	91	426	121	839
127	202	80 - 402		Oligospermia			363	103	370
133	88	11 - 159		195	216	21	321	280	685
144	112	60 - 246		Oligospermia			286	398	706

(*) Indicates subject dropped program before complete observations were made.

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TABLE III (Continued): EFFECT OF GRADED DOSES OF X-RAY, DELIVERED ACUTELY TO THE TESTES, ON HUMAN SPERM PRODUCTION (million per cc)

RV No.	Control Sperm Counts Mean	Control Sperm Counts Range	X-Ray Dose (f = 0.95	Observed Days of Azoospermia First Day	Observed Days of Azoospermia Last Day	Observed Days of Azoospermia Duration	First Day to Reach Control Range (*)	Highest Post Irradiation Sperm Count	Total Days of Observation After X-ray
79	322	183 - 411	100r	129	241	112	*	Azoospermic	300
38	122	22 - 210		107	198	91	555	78	1017
48	78	32 - 132		114	346	232	556	127	1101
42	80	24 - 194		124	-	-	*	Azoospermic	276
16	46	32 - 84		127	418	291	718	123	1220
28	42	8 - 89		113	225	112	596	82	737
3	157	81 - 343		152	213	61	703	227	973
32	75	39 - 128		140	203	128	521	239	1835
43	97	44 - 157		209	271	62	383	56	466
47	198	86 - 369		201	-	-	*	Azoospermic	222
6	59	38 - 89		145	472	327	955	137	1022
7	133	55 - 198		111	-	-	*	Azoospermic	118
4	67	16 - 229		88	297	209	668	121	2225
2	74	18 - 147		109	-	-	*	Azoospermic	151
164	63	8 - 115		141	253	112	*	Azoospermic	449
19	13	10 - 17		100	529	421	*	Azoospermic	682
71	68	3 - 142		In segregation		-	660	227	1036
120	68	30 - 128		189	301	112	556	73	904
165	63	15 - 153		-	-	-	*	Azoospermic	99
192	119	39 - 202		141	253	112	594	108	728
23	302	195 - 410	400r	116	458	342	*	51	948
14	59	33 - 79		115	444	329	983	104	1540
22	113	62 - 185		76	223	147	*	11	349
5	141	54 - 220		98	294	196	*	5	448
26	71	14 - 159		113	451	338	745	215	2544
57	78	22 - 153		-	-	-	*	Azoospermic	69

(*) Indicates subject dropped program before complete observations were made.

Histologic studies

The histologic studies, as outlined in our April 1, 1969 progress report, have now been completed. Table IV summarizes the normal germ cell population in a series of 27 control testicular biopsy specimens. The results are expressed as average counts per tubule. Considerable variation is seen in numbers of cell typesper tubule. This is as expected since the cells are not randomly distributed throughout the tubules, but are arranged in small constellations, or cellular association groupings. By counting 10 or more cross-sectional tubules, however, a relatively constant mean value can be established for any individual (Enclosure 3, Barr et al, J. Reprod. Fert. 25: 75-80, 1971). This has been demonstrated by having three trained independent observers count series of 40 randomly selected tubules from several different specimens. These results were then compared for between-observer agreement. The randomly-selected tubules for each specimen were then divided into groupings of ten tubules each. It was decided that for the critical cell types to be evaluated for this study quantitation of 10 tubules per specimen presented a representative sampling.

In collaboration with Dr. Oscar Vilar, we were able to make ultrastructural observations on the normal germinal epithelium, utilizing material from some of our control volunteers. These data were presented at the Workshop Conference on the Human Testis, Positano, Italy, in April 1970. (See Enclosure 4, Vilar, et al, from The Human Testis, Eds. Rosemberg and Paulsen, Plenum Press, New York-London, 1970, p. 63-74.)

Because of the large variation in cell types between individuals, it was desirable when possible to compare each man's post-irradiation biopsy specimen with his own control. Table V summarizes these pre- and post-irradiation comparisons. The leptotene-zygotene (LZ) and pachytene (P)

primary spermatocytes being the most numerous and, therefore, least variable of the cell types demonstrate most clearly the reduction and recovery of spermatogenic activity.

Restoration of the pachytene population to approximate control levels occurred, where observations were available, between the 600 to 900th day at all dose levels from 7.5 to 100r (Tables V and VI). At the 400r exposure level a return to the primary spermatocyte population control levels was noted between 1540 and 2191 days post-irradiation.

From the data presented in Tables V and VI the following changes can be summarized in the various germ-cell types at the time-intervals and dosages selected:

1. 7.5r showed a variable reduction in A-pale (Ap) spermatogonia and in pachytene (P) primary spermatocytes at intervals of from 433-730 days, although sperm production was not noticeably reduced (Table III).

2. 15r produced probable reductions in A-dark (Ad) spermatogonia and definite reductions in the A-pale population between 175 and 201 days, while the type B spermatogonia population remained decreased for approximately 300 days. The leptotene-zygotene (LZ) and pachytene primary spermatocytes remained decreased for over 300 days. This was reflected in the decreased sperm production of 3 out of 8 men (Table III), although azoospermia was not achieved.

3. 30r showed marked reductions in all spermatogonia and primary spermatocytes through 264 days. By 887 days the A-dark spermatogonia and the primary spermatocytes had started to show recovery.

4. 50r. After the 609th post-irradiation day the numbers of spermatogonia and primary spermatocytes had returned to normal and

remained so through day 1391, the last day of our observations.

5. 100r, for which most of the observations were made, was the lowest exposure used that consistently produced azoospermia. The type B spermatogonia, followed by their successors--the early primary spermatocytes--were the most quickly denuded. Apparently the B spermatogonia were the most radiosensitive and subject to the cell-kill mechanism discussed by Thorslund (Enclosure 2). This assumption is based on the fact that their numbers decreased, or disappeared almost immediately. The A-pale and A-dark appeared to be more subject to 'mitosis halting', or a failure to reproduce and proceed to the meiotic prophase stage of spermatogenesis. This concept explains the long quiescent period before repopulation of the A-dark then the A-pale and finally the B spermatogonia began to re-occur.

The fact that the A-dark spermatogonial population never actually disappeared supports the concept that this cell classification includes the primordial stem-cell line, and that this stem cell is the most radio-resistant of all spermatogonia.

Between approximately 225 to 466 days the A-dark spermatogonia had replenished their numbers, and spermatogenesis again began to proceed--the A-pale and B spermatogonia began to reappear in significant numbers, and by 737 days the primary spermatocyte population had been re-established.

6. 400r produced a more prompt and severe pattern of destruction of spermatogonia and subsequent disappearance of primary spermatocytes than seen at the 100r exposure level. Recovery was also delayed and the primitive A-dark spermatogonia did not consistently replenish their numbers until sometime between 1000 and 1540 days.

It is noteworthy that at all dose levels where cellular damage to the A-dark spermatogonia was observed, the progression of spermatogenesis was essentially halted beyond this stage. It was not until the stem-cell line had re-populated its numbers that spermatogenesis proceeded to the A-pale and B spermatogonia. From this point it was a matter of the normal time-sequence required for the primary spermatocytes to repopulate and for sperm to reappear in the ejaculate.

These observations are in agreement with the stem-cell renewal concepts of Clermont and Leblond (Am. J. Anat. 93:475-501, 1953) and Huckins (Anat. Rec. 169:553-558, 1971) for rodents. Huckins in particular, using whole mount and radioautographic technics, demonstrated intercellular bridges between spermatogonia of similar types. She showed progressive types to multiply in a logarithmic 2^{nth} fashion with clusters of 2-4-8 and 16 cells.

This fascinating study would be more difficult to demonstrate in man since, in contrast to rodent testes, a cross-section of any human seminiferous tubule contains from 2 to 5 small "constellations" or groupings of cell types. In rodents the so-called wave of spermatogenesis is much longer, and any given tubular cross-section contains only one such grouping, or stage of spermatogenesis. However, this basic process though more obscure, undoubtedly occurs in man.

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TABLE IV. NORMAL GERM-CELL POPULATION, AVERAGE COUNTS PER TUBULE

RV No.	Sertoli Cells	Spermatogonia A-dark (?)*	Spermatogonia A-Pale (16)*	Primary Spermatocytes Leptotene-Zygotene (7)*	Primary Spermatocytes Pachytene (16)*	Sperm Count (Million/cc) Mean	Sperm Count (Million/cc) Range
2	10.2	13.2	4.9	9.2	21.2	74	18-147
4	15.4	21.2	4.1	4.7	20.8	67	161-229
7	10.6	13.2	3.7	11.2	27.9	133	55-198
13	14.4	21.6	1.5	5.3	21.5	29	4- 87
14	16.4	17.8	3.5	9.2	47.2	59	33- 79
16	9.1	16.0	4.8	9.1	35.5	56	32- 84
17	15.0	24.8	14.1	10.5	35.0	60	12-186
18	20.4	16.8	7.2	8.5	28.1	163	82-286
23	14.2	19.1	3.9	7.9	41.6	302	195-410
26	11.1	12.2	0.9	8.6	23.8	69	26-125
30	11.4	17.2	2.1	6.8	18.9	113	62-185
31	12.0	14.2	6.5	12.4	28.9	220	80-416
32	13.0	7.8	17.7	14.0	37.0	75	39-128
38	18.1	18.2	6.2	19.0	37.3	122	22-210
39	17.4	9.8	9.3	7.2	40.4	239	136-427
41	23.3	18.2	9.5	16.3	42.1	324	199-652
42	15.4	12.7	5.8	13.8	32.7	80	24-194
43	17.2	19.2	5.7	12.8	32.1	97	44-157
47	16.2	20.3	4.6	11.9	41.2	198	86-369
48	12.5	15.2	5.5	12.6	19.6	70	32-132
53	13.9	11.8	5.9	10.3	37.7	147	82-206
59	13.5	15.8	1.5	13.6	25.1	73	23-145

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Continuation of Table IV

RV No.	Sertoli Cells	Spermatogonia A-dark (?)*	Spermatogonia A-Pale (16)*	Primary Spermatocytes Leptotene-Zygotene (7)*	Primary Spermatocytes Pachytene (16)*	Sperm Count (Million/cc) Mean	Sperm Count (Million/cc) Range
63	14.9	11.2	3.0	10.5	26.1	120	45-239
68	17.2	15.7	6.8	11.7	41.9	194	37-324
72	11.7	13.4	3.3	17.6	29.2	169	78-274
74	10.7	18.6	1.6	9.3	27.3	0	Vasectomized
101	15.3	17.2	2.8	8.3	31.6	272	175-617
Means	14.4	16.8	5.0	11.0	31.4		

(*) Digits with parentheses refer to life span, in days, for each cell type as estimated by Clermont.

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TABLE V. INDIVIDUAL CONTROL BIOPSY CELL COUNTS COMPARED WITH HIS POST-IRRADIATION VALUES

Rv	Days After Irradiation	Sertoli	A Dark	A Pale	Leptotene-Zygotene	Pachytene
15r		(14.4)*	(16.8)*	(5.0)*	(11.0)*	(31.4)*
39	Control	17.4	9.8	9.3	7.2	40.4
	201 days	16.3	10.3	1.8	4.5	21.0
41	Control	23.3	18.2	9.5	16.3	42.1
	305 days	25.6	11.9	5.3	8.1	16.9
18	Control	20.4	16.8	7.2	8.5	28.1
	306 days	18.9	15.5	5.8	9.3	18.0
50r						
17	Control	15.0	24.8	14.1	10.5	35.0
	609 days	19.6	17.8	14.8	13.2	26.4
	973 days	16.4	26.2	9.5	17.0	33.3
100r						
7	Control	10.6	13.2	3.7	11.2	27.9
	49 days	23.6	7.5	3.2	0.5	2.9
53	Control	13.9	11.8	5.9	10.3	37.7
	49 days	18.2	5.8	3.2	2.2	6.2
2	Control	10.2	13.2	4.9	9.2	21.2
	49 days	21.7	4.0	2.5	0.7	1.3
	153 days	18.4	1.0	0.4	0	0
47	Control	16.2	20.3	4.6	11.9	41.2
	33 days	22.2	10.2	3.5	0.2	3.9
	228 days	18.9	1.5	0.8	0	0
42	Control	15.4	12.7	5.8	13.8	32.7
	15 days	16.0	8.6	4.0	3.3	9.1
	280 days	16.9	5.5	1.9	0.4	3.0
43	Control	17.2	19.2	5.7	12.8	32.1
	33 days	21.8	12.5	6.5	1.9	8.9
	466 days	14.0	14.3	3.2	9.0	15.1
38	Control	18.1	18.2	6.2	19.0	37.3
	9 days	16.2	11.3	3.3	9.5	18.6
	1017 days	16.7	16.7	1.8	11.7	41.9

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Continuation of Table V

RV	Days After Irradiation	Sertoli	A Dark	A Pale	Leptotene-Zygotene	Pachytene
100r						
48	Control	12.5	15.2	5.5	12.6	19.6
	15 days	17.3	8.2	3.5	1.1	16.9
	1104 days	11.8	21.1	1.9	14.2	26.3
16	Control	9.1	16.0	4.8	9.1	35.5
	15 days	14.1	14.2	4.5	5.3	22.5
	1219 days	14.3	22.3	3.2	7.5	31.6
32	Control	13.0	7.8	17.7	14.0	37.0
	33 days	19.2	9.2	4.8	5.1	10.1
	1835 days	12.6	22.2	4.4	8.7	47.4
74	Control	10.7	18.6	1.6	9.3	27.3
	311 days	15.3	4.3	1.2	3.3	4.7
	1846 days	13.3	20.5	3.4	6.3	23.3
4	Control	15.4	21.2	4.1	4.7	20.8 (1)
	2225 days	14.4	22.3	3.0	4.1	13.8 (1)
400r						
23	Control	14.2	19.1	3.9	7.9	41.6
	967 days	22.4	0.5	0.2	0.2	1.3
14	Control	16.4	17.8	3.5	9.2	47.2
	91 days	25.9	0.3	0	0	0
	1540 days	17.8	15.9	2.5	13.5	33.3
26	Control	11.1	12.2	0.9	8.6	23.8
	503 days	16.1	3.3	1.5	5.5	6.4
	2191 days	13.3	16.1	0.4	12.6	24.1

(*) Digits in parenthesis refer to average counts per tubule.

(1) RV 4 is included, although his recovery was complicated by an unrelated cerebral vasculitis and septicemia, secondary to an infected pilonidal cyst. This makes conclusions uncertain.

TABLE VI. POST-IRRADIATION CELL COUNTS RELATED TO DOSE AND RECOVERY TIME INTERVALS

X-ray Dose	RV No.	Days After Irradiation	Spermatogonia S	AD	AP	B	Primary Spermatocytes PL	LZ	P
Mean Counts per Tubule of 27 Normal Controls ± S.D.			14.4 3.2	16.8 3.8	5.0 2.9	3.1 1.1	1.5 0.97	11.0 3.4	31.4 7.9
7.5r	13	433	12.8	21.5	5.5	2.4	1.7	8.9	16.9
	99	622	15.3	28.5	2.6	2.7	1.8	22.6	43.2
	141	730	15.6	26.3	1.7	2.5	0.9	8.3	19.6
15r	154	175	15.6	7.8	0.2	1.1	0.3	1.2	5.0
	39	201	16.3	10.3	1.8	1.9	0.1	4.5	21.0
	41	305	25.6	11.9	5.3	1.6	0.8	8.1	16.9
	18	306	18.9	15.5	5.8	2.3	1.0	9.3	18.0
30r	21	159	25.3	2.6	2.0	0.5	0	2.4	1.3
	30	222	17.9	3.5	1.5	0.4	0	2.7	4.6
	34	264	19.0	6.7	2.9	1.1	0	3.1	7.2
	29	887	14.9	12.8	2.8	-	-	11.5	24.0
50r	17	609	19.6	17.8	14.8	3.3	1.7	13.2	26.4
	17	973	16.4	26.2	9.5	3.0	0	17.0	33.3
	8	1391	13.1	24.6	2.7	3.8	1.5	17.9	37.7
100r	79	Immediately	17.0	11.8	2.9	-	-	14.8	35.1
	38	9	16.2	11.3	3.3	0.6	0	9.5	18.6
	54	14	23.1	13.3	5.2	1.5	1.5	5.8	23.5
	48	15	17.3	8.2	3.5	1.3	0.9	1.1	16.9
	42	15	16.0	8.6	4.0	1.0	0.4	3.3	9.1
	16	15	14.1	14.2	4.5	0.9	0.3	5.3	22.5
	28	24	18.0	8.7	5.1	0.7	0	4.5	11.9
	27	27	13.7	7.7	5.3	1.1	0	1.0	5.2

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Continuation of Table VI

X-ray Dose	RV No.	Days After Irradiation	Spermatogonia S	AD	AP	B	Primary Spermatocytes PL	LZ	P
100r	32	33	19.2	9.2	4.8	0.8	1.7	5.1	10.1
	43	33	21.8	12.5	6.5	1.5	0.7	1.9	8.9
	47	33	22.2	10.2	3.5	1.0	0	0.2	3.9
	53	49	18.2	5.8	3.2	0.4	0	2.2	6.2
	7	49	23.6	7.5	3.2	0.7	0	0.5	2.9
	2	49	21.7	4.0	2.5	0.5	0	0.7	1.3
	2	153	18.4	1.0	0.4	0	0	0	0
	164	182	18.6	2.7	0	0	0	0.2	0.4
	47	228	18.9	1.5	0.8	0.1	0	0	0
	42	280	16.9	5.5	1.9	1.0	0.2	0.4	3.0
	74	311	15.3	4.3	1.2	-	-	3.3	4.7
	43	466	14.0	14.3	3.2	-	-	9.0	15.1
	28	737	12.4	9.5	5.8	-	-	16.9	40.0
	149	819	17.7	19.4	1.8	2.2	1.8	13.9	32.3
	3	973	11.3	14.9	4.8			16.9	32.6
	38	1017	16.7	16.7	1.8	2.8	0.6	11.7	41.9
	6	1022	13.2	16.4	5.6	-	-	15.7	29.6
	48	1104	11.8	21.1	1.9	2.8	1.3	14.2	26.3
	16	1219	14.3	22.3	3.2	4.0	2.9	7.5	31.6
	32	1835	12.6	22.2	4.4	5.1	1.5	8.7	47.4
	74	1846	13.3	20.5	3.4	2.9	2.3	6.3	23.3
	4	2225	14.4	22.3	3.0	1.9	3.0	4.1	13.8 (1)

(1) RV 4 is included, although his recovery was complicated by an unrelated cerebral vasculitis and septicemia, secondary to an infected pilonidal cyst. This makes conclusions uncertain.

Continuation of Table VI

X-ray Dose	RV No.	Days After Irradiation	Spermatogonia S	AD	AP	B	Primary Spermatocytes PL	LZ	P
400r	23	Immediately	11.9	13.0	6.1	3.0	1.3	12.3	27.9
	63	Immediately	15.7	10.9	3.8	-	-	6.8	12.6
	14	91	25.9	0.3	0	0	0	0	0
	22	188	23.3	1.1	1.1	0.3	0	0.6	0.8
	22	355	20.2	6.6	2.1	1.5	0.7	3.4	7.9
	5	450	15.0	2.7	1.2	0.1	0	2.4	7.9
	26	503	16.1	3.3	1.5	-	-	5.5	6.4
	23	967	22.4	0.5	0.2	0	0	0.2	1.3
	14	1540	17.8	15.9	2.5	2.8	0.3	13.5	33.3
	26	2191	13.3	16.1	0.4	2.8	1.5	12.6	24.1

3002856

Seminiferous Tubular Wall and Vascular Analysis

We referred in our previous request for renewal of AEC Contract No. AT(45-1)-225, Task Agreement No. 6, page 2, to a long-term study which had been made of survivors of the Hiroshima and Nagasaki explosions in 1945 (ABCC Technical Report 15-66, and Archives of Pathology 82:542-54, 1966). In that study, made years later at autopsy, the testes of those survivors who had been within 1400 meters from the hypocenter and who had received an estimated exposure of 300 or more rads were compared with a similar age group who had been in distant Japanese cities at the time of the explosions.

Their findings indicated that there was an increase in the prevalence of seminiferous tubular sclerosis and vascular hyalinization in the testes of those men exposed to irradiation as compared with the control group.

Since we knew precisely the rad-dose we delivered to the testes, we re-evaluated all biopsy specimens from each volunteer for whom both control and post-irradiation biopsy specimens were available, as it is important to establish whether or not a time- or dose-response relationship exists for either tubular wall or vascular (arteriolar or venous) wall damage.

The data for tubular wall damage are shown in Table VII (see Plate I for histologic examples). The criteria for seminiferous tubular wall damage was based on a scale of '0' to '3' as follows:

0 = 1 micron basement membrane diameter with normal germinal epithelium

1 = 2 micra basement membrane diameter with normal germinal epithelium

2 = 3-9 micra basement membrane with some germinal and Sertoli cells present

3 = 5-13 micra basement membrane diameter with virtually no intra-tubular cells present

Classification '0' is always present in the testes of normal men; classification '1' is found in varying numbers in many normal testicular specimens, and unless a high percentage of these are seen it is not considered to be abnormal; classification '2' represents moderate to severe damage, even though complete but reduced spermatogenesis may be present, while classification '3' indicates complete or almost complete atrophy of the tubule.

The classification of vascular wall damage was more subjective, but again a scale of '0' to '3' was used--normal, minimal, moderate and severe damage. (See Plate II for examples).

The vascular wall evaluation revealed no significant alterations. After careful examination of all control and post-irradiation biopsy specimens, we were able to find one or two vascular plaques in one individual 6 years after exposure to 400r x-ray. We had previously reported seeing this at a lower dose level (100r). However, it should be noted that we do not know the significance of these findings since we occasionally observe isolated vascular changes in an otherwise 'normal' testis.

In contrast, the basement membrane of the seminiferous tubules generally showed an increased thickening during the early phase of germ-cell denudation (Table VII). Since much of this early thickening regressed to Classification '0' as spermatogenesis recovered it seems likely that in part this represents a natural contraction, or involution of the membrane structure during the period of germ-cell loss. This contraction, however, cannot completely explain the increased percentage in thickening, as in some instances the percentages of '1', '2' and even '3' classifications remained increased long after spermatogenesis had been restored, as shown in Tables V, VII and VIII.

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It is generally concluded by gonadal investigators that the average Sertoli cell population per tubule is relatively constant for any given individual, and many have used the Sertoli-cell/germ-cell ratio as a means of evaluating spermatogenesis. In the event of tubular wall thickening there is probably a decrease in the ability of nutrients to pass the basement membrane barrier and to sustain both the Sertoli cells as well as the germinal epithelium. Thus Table VIII gives examples of tubular wall classifications and the respective numbers of Sertoli cells and pachytene primary spermatocytes found per tubule in the different classifications and time intervals following irradiation. Although the number of tubules observed for the 'increased thickening' classifications is quite small, the correspondingly progressive decrease in both Sertoli cells and primary spermatocytes suggests that some degree of this persistent basement membrane damage had occurred; furthermore, a correlated decrease in both Sertoli cells and primary spermatocytes was observed in scattered areas of the post-irradiated testis.

In summary, it must be noted that our data are not as exhaustive as the Japanese study--nor of sufficient time duration-- to make conclusions with respect to vascular damage. The basement membrane changes, however, are interpretable. This structure seems to be somewhat radio-sensitive. In some instances the thickening induced by irradiation disappeared, whereas in other situations the thickening has persisted. Therefore, these data with regard to seminiferous tubular wall changes following acute irradiation to the testes lends support to the Hiroshima-Nagasaki study.

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TABLE VII. SUMMARY OF X-RAY INDUCED SEMINIFEROUS TUBULAR WALL MEMBRANE DAMAGE

X-ray Dose	RV No.	Control Biopsy and Days After Irradiation	Percentage of Seminiferous Tubules Showing Damage with Respect to Time & Classification 0	1	2	3
50r	8	0	37.5	46.9	15.6	0
		1390	70.5	15.9	6.8	6.8
	17	0	83.3	12.5	4.2	0
		609	65.6	21.9	12.5	0
		973	57.1	14.3	17.1	11.4
100r	2	0	100	0	0	0
		49	75.5	24.5	0	0
		153	75.5	24.5	0	0
	3	0	100	0	0	0
		973	0	88.9	11.1	0
	6	0	no control			
		1022	95.6	4.4	0	0
	7	0	53.8	30.8	15.4	0
		49	0	91.3	8.7	0
	16	0	92.1	7.9	0	0
		15	0	83.3	16.7	0
		1219	0	75.0	25.0	0
	27	0	80.0	20.0	0	0
		27	0	100	0	0
		1050	88.0	12.0	0	0
	28	0	97.2	2.8	0	0
		24	78.3	21.7	0	0
		737	100	0	0	0
	32	0	82.1	10.7	3.6	0
		33	80.0	13.3	4.0	2.7
		1115	0	100	0	0
	38	0	91.2	8.8	0	0
		9	71.2	25.0	3.8	0
		1017	57.1	33.3	9.5	0
	42	0	61.5	26.9	11.5	0
		15	34.3	47.1	15.7	2.9

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Continuation of Table VII

X-ray Dose	RV No.	Control Biopsy and Days After Irradiation	Tubular Wall Classification 0	1	2	3
100r	43	0 right side	90.5	6.3	3.2	0
		0 left side	72.5	20.0	5.0	2.5
		33	100	0	0	0
		466	90.0	10.0	0	0
	47	0	72.7	18.2	3.9	5.2
		33	34.4	40.6	25.0	0
		228	40.0	40.0	12.7	7.3
	48	0	58.3	33.3	8.3	0
		15	40.7	48.2	7.4	3.7
	53	0	76.6	20.8	1.3	1.3
		49	71.4	23.0	5.6	0
	54	0	84.8	10.9	4.3	0
		14	62.5	31.3	6.2	0
	74	0	76.8	10.1	4.4	8.7
		308	44.9	50.0	5.1	0
		1848	42.0	54.8	3.2	0
	79	0 right side	68.7	18.1	13.2	0
		0 left side	76.4	16.4	7.3	0
		4 hours	80.4	16.1	1.8	1.8
400r	5	0	100	0	0	0
		450	0	100	0	0
	14	0	91.4	8.6	0	0
		91	7.5	92.5	0	0
		1539	82.4	14.7	2.9	0
	22	0	100	0	0	0
		188	91.2	8.8	0	0
		355 right side	93.6	3.7	0	0
		355 left side	93.5	6.5	0	0
	23	0	100	0	0	0
		4 hours	88.5	11.5	0	0
		967	62.5	37.5	0	0
	26	0	98.0	0	2.0	0
		504	80.7	14.9	4.4	0
		2192	63.6	33.3	1.0	0

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Continuation of Table VII

X-ray Dose	RV No.	Control Biopsy and Days After Irradiation	Tubular Wall Classification 0	1	2	3
400r	57	0 left side	95.5	4.5	0	0
		0 right side	97.8	2.2	0	0
	63	0 left side	100	0	0	0
		0 right side	90.5	9.5	0	0
		4 hours	93.1	6.9	0	0
		63	0	100	0	0
	83	0 (year 1966)	100	0	0	0
		0 (year 1967)	0	100	0	0
		4 hours	0	93.5	6.5	0
		322	0	83.3	16.7	0

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TABLE VIII. COMPARISON OF TUBULAR WALL THICKENING WITH SERTOLI CELL AND PRIMARY SPERMATOCYTE POPULATIONS

X-ray Exposure	Subject	Days After Irradiation	Tubular Wall Classification	Sertoli Cells: Number of Tubules Found Classification	Sertoli Cells: Number Cells Per Tubule	Pachytene Primary Spermatocytes: Number of Cells Per Tubule
50r	RV 17		0	15	13.8	33.0
		control	1	1	15.0	35.0
			2	1	11.0	8.0
			3	0	0	0
			0	11	13.4	39.3
		609	1	6	14.5	32.2
			2	2	5.5	11.5
			3	0	0	0
			0	7	14.3	40.9
		973	1	7	7.9	21.1
			2	3	7.0	0
			3	2	0	0
100r	RV 32		0	67	13.3	41.8
		control	1	2	10.0	15.5
			2	1	1.0	0
			3	2	0	0
			0	100	14.6	10.1
		33	1	21	14.9	2.4
			2	3	12.0	5.0
			3	3	0	0
			0	2	12.0	51
		1115	1	1	6.5	30
			2	5	0	0
			3	5	0	0
400r	RV 14		0	15	14.5	38.7
		control	1	2	12.0	33.0
			2	2	4.0	4.5
			3	1	0	0
			0	4	18.2	0
		91	1	18	17.8	0
			2	1	0	0
			3	1	0	0
			0	7	12.6	29.4
		1539	1	4	21.0	7.2
			2	2	4.0	3.0
			3	0	0	0

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PittsburghRadiation Accident

In our progress report of March 10, 1970, we reported on the opportunity to make a practical application of information gained from our in-progress radiation studies. This involved a study of an accident which occurred in Pittsburgh, Pennsylvania, on October 4, 1967. Three invidivuals were exposed to x-rays which eminated from the gold target of a linear accelerator that possessed a median energy of 300 KVP and a maximum energy of 3 MEV. The film badge readings were as follows: Patient No. 1, 550r; Patient No. 2, 550r, and Patient No. 3, 110r.

Patient No. 2 sustained doses estimated to be at least 1000r to several areas of his body including his hands and feet. The acute exposures to the testes, of course, could only be estimated by reconstruction, after using after-the-fact mock-up technics. Immediate intensive care and follow-up studies were initiated. Patient No. 2, who had the most severe total body exposure, fortunately had an identical twin and on October 12--8 days later the twin was able to provide a successful bone marrow transplant. Hematopoetic recovery began 12 days later and from data compiled by Dr. E. Donnall Thomas, et al (Exp. Hematology 21:16-18, 1971) his platelet and white blood cell counts repopulated at a much faster rate than that observed in a comparable individual from another study who had received 400 rads, with no marrow transplant.

We have now followed gonadotrophin (FSH and LH) levels, and where possible, seminal fluid analyses in these men for six years. Table IX of this report brings Table III of the March 10, 1970, progress report to its current status. Patient No. 2 was unable to submit seminal fluid specimens due to the loss of both hands following the exposure. Patient No. 3, who

received an estimated 100r, returned to acceptable sperm count levels at about 500 days. Patient No. 1, who received an estimated 550r, returned to a satisfactory sperm count range at about 1000 days after the accident.

Our previously mentioned volunteer data using calibrated dose levels have provided valuable information as to the probable rad exposure range sustained by the testes of these three men. From our data we conclude that probable gonadal exposures were within the calculated dose ranges; however, the fact that the serum FSH levels for Patient No. 2 remained elevated for a much more extended period of time indicate that he sustained considerably higher exposure to his testes than did Patient No. 1. It also should be noted that both the FSH and LH levels are within the normal range for all three men as of the last report.

TABLE IX. EVALUATION OF PITTSBURGH RADIATION ACCIDENT

Date	Days After X-ray	Patient No. 1(550r)			Patient No. 2(550r)			Patient No. 3(110r)		
		SF+	FSH	LH	SF	FSH	LH	SF	FSH	LH
1968										
Feb	124	0						<1.0		
Mar	148	0						0		
Apr	182	0						0		
May	210	0	41.0*					1.0	55.0*	
June	245	0	18.0					1.0	66.7	
July	273	0	11.7					2.5	61.0	
Aug	315	0	18.7					7.7	37.4	
Sept	336	0	18.8			14.3		12.4	47.8	
Oct	364	0	28.4			12.9		10.7	48.8	
Nov.	399	-	-			16.0		-	-	
Dec	427	0.5	11.8			17.2		25.0	43.8	
1969										
Jan	462	0.8	19.2			22.9		33.0	49.6	
Feb	490	1.0	15.6			16.2		28.0	23.0	
Mar	518	2.8	-			6.6		58.0	27.0	
Apr	546	3.9	24.0			14.3		48.0	45.0	
May	581	5.8	16.8			-		30.0	14.0	
June	609	6.5	20.0	13.0**		-		48.0	15.0	7.6**
July	644	3.3	340**	12.5		-		50.0	17.6	
Aug	672	9.1	270	-		-		54.0	375**	5.1
Sept	700	20.0	270	7.9		480**		35.0	380	3.8
Oct	728	33.0	370	5.4		720		43.0	560	9.4
Nov	763	40.0	375	9.5		760	24.0**	32.0	540	3.6
Dec	791	46.0	355	5.6		780	20.0	73.0	450	4.1
1970										
Jan	826	59.0	370	8.4		860	34.0	38.0	370	8.2
Feb	854	66.0				600	14.0	51.0	435	9.4
Mar	882	16	280	7.6		590	18.5	51	455	6.5
Apr	913	34	315	9.0		700	25.0	49	460	5.5
May	948	7.3	340	7.5		640	14.0	17	480	6.5
June	976	43	420	2.5				38		
July	1004		415	9.5		778	19.2		480	8.0
Aug	1039	121	402	5.8				34	320	6.4
Sept	1067		335	8.6		565	23.0		410	13.2
Oct	1102		457	4.3		1090	18.5		700	7.8
Nov	1130		440	7.1		850	16.0		570	10.5
Dec										
1971										
Jan	1193		340	6.9		502	17.4		408	6.6
Feb	1221		313	4.6		525	17.8		450	9.5
Mar	1243		325	5.3		540	27.1		448	9.1
Apr	1278		295	6.8		500	17.6		328	3.9
May	1306		270	4.5		775	30.7		510	8.4

PRIVACY ACT MATERIAL REMOVED

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Continuation of Table IX

Date	Days After X-ray	Patient No. 1(550r)			Patient No. 2(550r)			Patient No. 3(110r)		
		SF+	FSH	LH	SF	FSH	LH	SF	FSH	LH
1971										
June										
July										
Aug										
Sept	1425		253	3.6		790	25.0		570	9.3
Oct	1460		250			735			430	
Nov	1488		223			735			437	
Dec	1516		232			590			430	
1972										
4-5	1642		159	5.6		410	19.0		247	13.6
5-31	1698		192	6.1						
9-6	1796		129	6.1		410	19.8		216	16.3

+SF - Seminal fluid sperm count in million/cc
* Urinary FSH, normal range <1.8 - 25.0 IU
** Serum FSH, normal range 230-600 mμg Ler 907/cc, by radioimmunoassay.

** Serum LH, normals range 4-19 mIU/ml, by radioimmunoassay

PRIVACY ACT MATERIAL REMOVED

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FINAL SUMMARY

The studies and conclusions derived from this study may be briefly summarized as follows:

1. We were able to develop a rapid and efficient method for automatic counting of large numbers of seminal fluid samples, which compared precisely with the visual hemacytometer method (r = .99). The success of this Coulter counter method depended on developing a diluent that would not only lyse white cells, but would also dissolve the crystals and prostatic inclusion granules normally found in seminal fluid. (Gordon, et al. RLO-1781-1, and Gordon, et al. RLO-1781-5)

2. Fundamental data have been gathered on the inherent individual variation in normal control sperm counts, as well as a fuller appreciation of the sensitivity of the testis to stresses such as testicular biopsy (Gordon, et al. RLO-1781-2). It was further found through many attempts and different approaches by numerous statisticians that conventional statistical methods cannot be applied to sperm-count analyses (Thorslund & Paulsen, Enclosure 2).

3. Sperm production was unaffected by acute x-ray exposure of 7.5r to the testes. A significant reduction was noted in 3 of 8 men following a dose of 15r. Exposure to 30r produced temporary oligospermia in 2 of 4 men, and 50r resulted in temporary oligospermia in 5 of 8 men. As noted in the text, our criteria for oligospermia is a temporary though consistent reduction in sperm count to less than 5m/cc. Doses of 100r and 400r produced temporary azoospermia in all men for whom adequate follow-up studies were available. In general the magnitude of the dose correlated with the rapidity of drop in sperm count and the duration of azoospermia, but there were wide overlaps. All men who were followed for adequate

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periods of time (Table III) exhibited a return to full spermatogenesis.

4. A. Germ Cells. Histologically, the type B spermatogonia seemed to be the most sensitive to immediate cell-kill, with the A-pale spermatogonia being the next most sensitive. The A-dark type spermatogonia, which presumably contains the primordial stem-cell line (see Huckins, referred to in the text) were the most radioresistant--although most appeared to die later or at least became mitotically inactive and failed to repopulate their numbers or to proceed through mitotic divisions to produce the A-pale and B type spermatogonia. It was of interest that after this quiescent period when re-population of the germinal epithelium did occur the A-dark cells re-established their normal cell numbers before proceeding to replenish the A-pale and B spermatogonia. When the type B spermatogonial population approached normal levels meiotic prophase of primary spermatocytes reinitiated and proceeded according to the time schedule established by Clermont, until mature sperm were again seen in the ejaculate. Both the primary spermatocytes and spermatids were resistant to direct damage by the x-ray dosages used.

B. Membranes and Vasculature. Analysis of control and recovery biopsy specimens indicate a tendency toward scattered residual thickening of the basement membrane of the seminiferous tubule. This finding supports studies from survivors of the Hiroshima-Nagasaki atomic explosions. No conclusions could be drawn regarding residual vascular wall damage. No changes in numbers or appearance of the interstitial cells of Leydig were noted at any dose level.

5. The hormone responses to acute x-ray exposures may be summarized as follows:

a. Serum testosterone levels did not change following any

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dosage used.

b. Some instances of transient elevations in LH were seen, but these fell within the ranges of normal day-to-day variation.

c. 15r was the lowest dose to cause a temporary increase in urinary FSH levels. The apparent dichotomy between this and the failure of this dose to produce azoospermia supports Dr. Leonard's hypothesis of an 'independent station' (Enclosure I).

d. Temporary elevated urinary FSH levels were also noted at the 30r and 50r dose level, even though azoospermia was not produced. This again supports the 'independent station' concept.

e. At the 100r and 400r exposure levels the elevations in urinary FSH titers were consistent with the observed azoospermia. As was mentioned in discussing sperm production, all men who were followed for a sufficient period of time to make conclusions, returned to their control FSH levels as spermatogenic activity returned to pre-irradiation levels.

f. The study originally utilized the general urinary gonadotrophin bioassay (GGA) which measures both FSH and LH. We soon adopted the Steelman-Pohley bioassay methods, which is specific for urinary FSH titers. Appropriate standards were used for both assay systems and the kinetics (correction factors, etc.) of the two methods were carefully evaluated before drawing conclusions regarding changes in FSH excretion levels.

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Publications supported by this grant

AEC support has been directly involved in each of the publications and presentations listed below, although some are not a part of the AEC contract program. For example, establishment of the chromosome laboratory enabled us to study other aspects of reproductive physiology. Likewise, establishment of the radioimmunoassay and subsequent purchase of the gamma counter allowed us to utilize radioimmunoassay in other endocrine studies as well as in studies directly supported by this contract. Therefore, appropriate credit has been given to the contract for each of the following:

RLO-1781-1 The Determination of Size and Concentration of Human Sperm with an Electronic Particle Counter. D.L. Gordon, D.J. Moore, T. Thorslund and C.A. Paulsen. The Journal of Laboratory and Clinical Medicine, March 1965.

RLO-1781-2 Testicular Biopsy in Man. I. Effect upon Sperm Concentration. D.L. Gordon, Arnold B. Barr, J.E. Herrigel, and C.A. Paulsen, Fertility and Sterility, July 1965.

RLO-1781-3 Effect of X-Irradiation of Testis on Plasma Testosterone in Normal Male Volunteers (abst). C.A. Paulsen, D.L. Gordon, G.R. Halling, A. Barr and H.M. Gandy. Submitted but not presented, Second International Congress on Hormonal Steroids, Milan, Italy, May 1966.

RLO-1781-4 The Effects of X-Ray Irradiation on the Human Testis (abst). C.A. Paulsen. Society for the Study of Fertility, Cambridge, England, July 1966.

RLO-1781-5 Efficacy of Coulter Counter in Determining Low Sperm Concentrations. D.L. Gordon, J.E. Herrigel, D.J. Moore, and C.A. Paulsen. American Journal of Clinical Pathology, January 1967.

RLO-1781-6 Third Year Comprehensive Progress Report, Contract AT(45-1)-1781 March 1966.

RLO-1781-7 Premature Menopause XO/XX/XXX/XXXXX Mosaicism. D.L. Gordon, and C.A. Paulsen. American Journal of Obstetrics and Gynecology, January 1967.

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RLO-1781-8 Klinefelter's Syndrome and Its Variants: A Hormonal and Chromosomal Study. C.A. Paulsen, D.L. Gordon, Richard W. Carpenter, H.M.Gandy, and W.D. Drucker. Recent Progress in Hormone Research, Vol. 24, 1968.

RLO-1781-9 Metabolism of Labelled ^{125}I-HCG by the Rat Ovary. D.H. Espeland, F. Naftolin, and C.A. Paulsen. Gonadotropins 1968, ed. E. Rosemberg, Geron-X, 1968.

RLO-1781-10 Comprehensive Progress Report, April 1969.

RLO-1781-11 Progress Report, An Addendum to Comprehensive Progress Report of April 14, 1969. Prepared March 10, 1970.

RLO-1781-12 Plasma and Urinary Follicle-Stimulating Hormone Levels in Oligospermia. John M. Leonard, Robert B. Leach, Margaret Couture, and C. Alvin Paulsen. J. Clin. Endocr. 34:209-14, 1972.

RLO-1781-13 Effects of X-Ray Irradiation on Human Spermatogenesis. T.W. Thorslund and C.A. Paulsen. From, Proceedings of the National Symposium on Natural and Man-Made Radiation in Space. Ed. E.A. Warman, NASA Document NAS No. 2440, Jan. 1972, pp. 229-232.

RLO-1781-14 Germinal Cell Loss During Human Spermatogenesis. Arnold B. Barr, Donald J. Moore and C. Alvin Paulsen. J. Reprod. Fert. 25: 75-80, 1971.

RLO-1781-15 Electron Microscopy of the Human Seminiferous Tubules. Oscar Vilar, C. Alvin Paulsen and Donald J. Moore. From: The Human Testis, Eds. Eugenia Rosemberg and C. Alvin Paulsen, Plenum Press, New York-London, 1970.

RLO-1781-16 Effects of HCG, HMG, HLH and HGH Administration on Testicular Function. C. Alvin Paulsen, Duane H. Espeland and Edward L. Michals. From: The Human Testis, Eds. Eugenia Rosemberg and C. Alvin Paulsen, Plenum Press, New York-London, 1970.

RLO-2225-1 Progress Report and Request for Renewal (Terminal Period) 1970-71.

RLO-2225-2 Final Progress Report of AEC Contract No. AT(45-1)-2225, Task Agreement No. 6, January 31, 1973.

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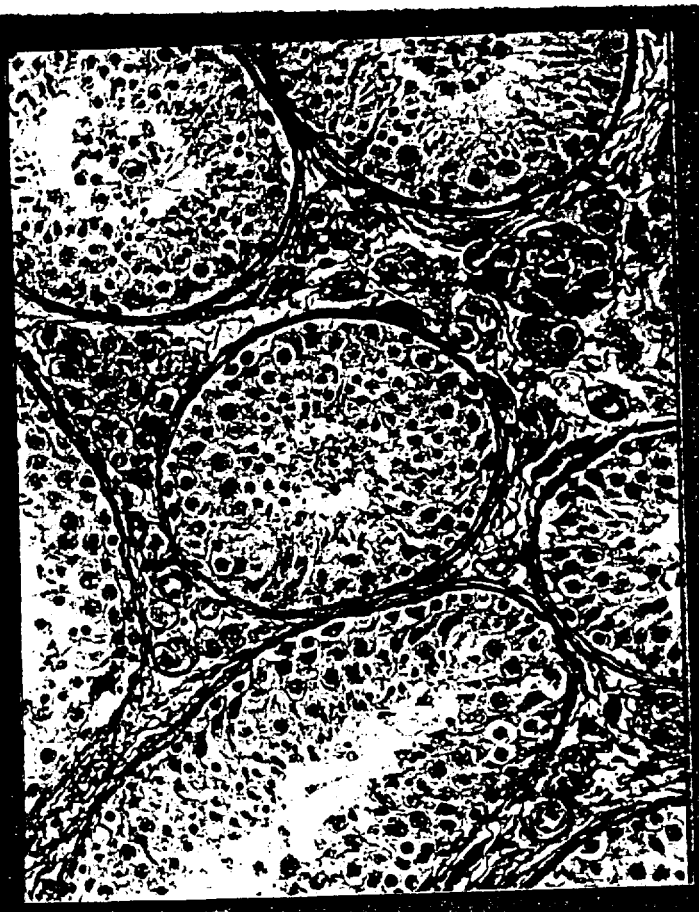
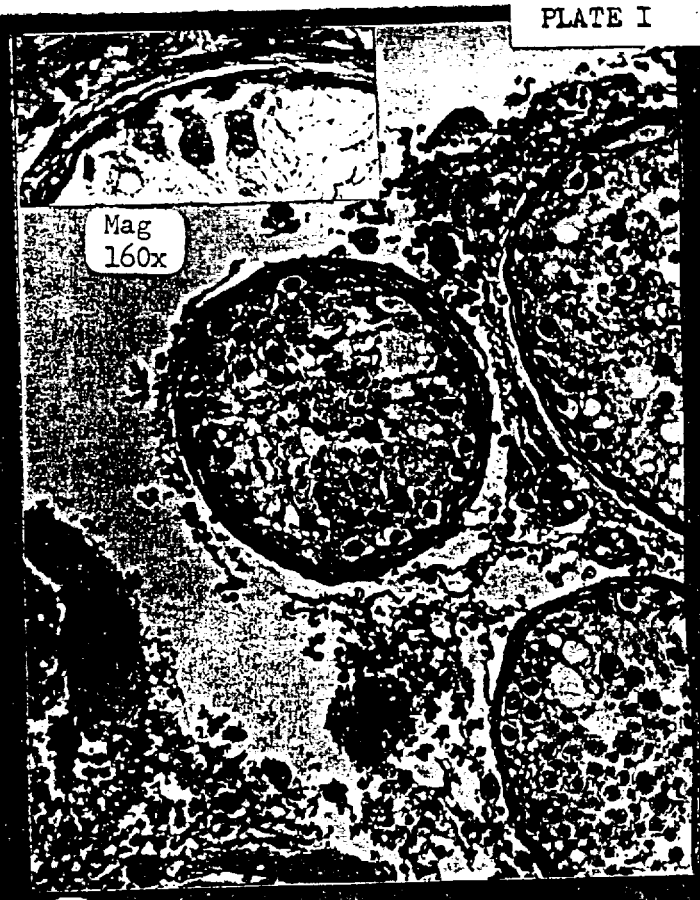
PLATE I



Classification '0' mag. 64x



Classification '1' mag. 64x

Classification '2' mag. 160x

Classification '3' 160x

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PLATE II LEGEND

Classification '0' shows a small testicular arteriole. The fine, blue-staining tunica intima is considered normal.

Classification '1' shows a smaller arteriole, but the increased thickness of the tunica intima, and perhaps some increase in hyaline or collagen material in the surrounding layers (blue staining) suggests minor damage.

Classification '2' shows an arteriole cut obliquely; however there is definite evidence of plaque-like formations in the intimal layer, as well as increased collagen material in the surrounding structures.

Classification '3' shows a large, occlusive collagenous plaque within the vessel as well as evidence of increased hyalinization, or collagenous material in the surrounding layers.

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Vascular Classification '0'
160x

Vascular Classification '1'
mag. 160x

Vascular Classification '2'
mag. 160x

Vascular Classification '3'
mag. 160x

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