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RADIATION RESEARCH 59, 665-678 (1974)

Effect of Graded Doses of Ionizing Radiation on the Human Testis¹

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ROWLEY, M. J., LEACH, D. R., WARNER, G. A., and HELLER, C. G. Effect of Graded Doses of Ionizing Radiation on the Human Testis. *Radial. Res.* 59, 665-678 (1974).

A portable unit was developed to provide uniform irradiation of the human testes. The device had built-in radiological protection and provided a dosage independent of the subject geometry, uniform to within $\pm 5\%$. Single doses, between 8 and 600 rad were administered to the testes of human subjects. Observations were made both before and following irradiation. Parameters evaluated included sperm concentration, motility and morphology, seminal fluid volume, plasma and urinary gonadotropin and testosterone levels, urinary estrogens, and comparison of testicular biopsies taken before and after irradiation in the same subject. Dose-response relationships and recovery times were determined for each dose range studied.

INTRODUCTION

During the past 10 yr (1963-1973) a long-term study on the effects of acute doses of X-ray irradiation on the normal human testis has been performed. Only preliminary publication of pertinent data has been made to date (1-5). Because of the extensive amount of data accumulated a series of reports is being written. This one, the first, will explain the details of the irradiations, the experimental design, and give a general over-view of the findings. Subsequent reports will provide detailed analyses of each of the parameters studied.

MATERIALS AND METHODS

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

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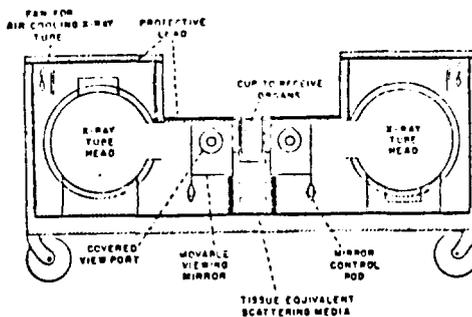


Fig. 1. Section through irradiation unit.

from 115 V 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 R/min.

The equipment provided (Figs. 1 and 2) consisted of two air-cooled industrial X-ray units, with tungsten targets, operating at approximately 140 kVp with up to 5 mA tube current, having an inherent filtration of 0.14 in. glass plus 0.12 in. Mg, equivalent to 2 mm Al. 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 $20 \times 20 \times 6$ cm thick, placed midway between the tubes. Inserted into this block of material in the middle of the upper edge was a transparent $10 \times 15 \times 6$ cm vessel of 0.125 in. Plexiglass.

In use the Plexiglass vessel was filled with warm ($93-94^\circ\text{F}$) 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

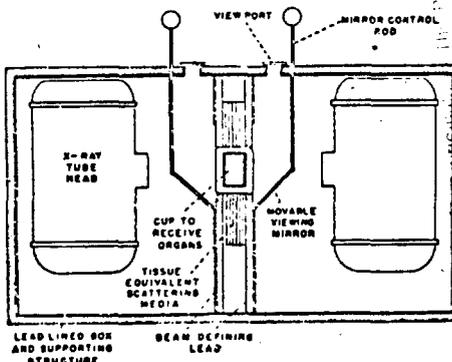


Fig. 2. Plan of irradiation unit from top view.

Fig. 3 (solid line)

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HUMAN TESTICULAR IRRADIATION

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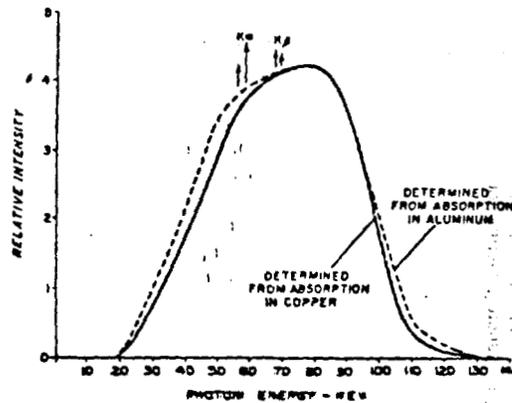


FIG. 3. Spectral distribution of the radiation as determined from absorption curves in copper (solid line) and aluminum (broken line).

material one tissue equivalent mass, thereby making the dose distributions in the testes independent of the natural variations in the geometry of the subject.

Characteristics of the X-ray heads were determined before assembly into the unit using a Victoreen "R" meter model 70 bearing calibrations against a free air chamber and a secondary standard certified by the National Bureau of Standards. They were found to deliver dose rates that were within 1% of one another when operated under the same conditions. Narrow beam absorption studies showed them to have first HVLs of 5 mm of aluminum or 0.25 mm copper, and second HVLs of 7.95 mm aluminum or 0.43 mm of copper. The peak kilovoltage as determined from the absorption curve in copper combined with data from Morgan (6) was found to be 135 kVp. The spectral distribution as determined from the absorption curves in aluminum and copper analyzed by the method of Greening (7) is shown in Fig. 3.

The X-ray heads were then assembled into the unit and the dose distribution explored with an air equivalent ionization chamber rate meter system of a type previously described (8). The chamber built for this project was wave length independent at the energies of interest and calibrated by comparison with the Victoreen "R" meter. The irradiation of a large volume compared with the volume of interest reduced the "wasting effect" usually observed in media irradiated by parallel opposing fields. The total size of the irradiated volume, the point of incidence of the central axis of the X-ray beams and the focus to subject distance were manipulated to obtain uniformity of exposure dose rate. Measurements showed that the dose rate was within $\pm 5\%$ of the average throughout the volume of the Plexiglass vessel, to within 1.7 cm of the uppermost surface of the vessel. Greater uniformity may be achieved by the use of wedge filters if needed. In use, however, irradiation was only performed when the testes descended below the 1.7 cm level as viewed in the mirrors

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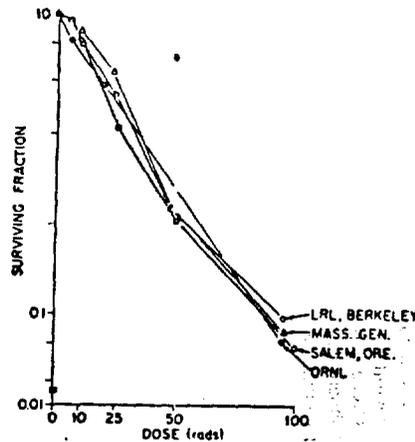


Fig. 4. Comparison of mouse spermatogonial survival with data from type A and intermediate spermatogonia pooled. Surviving fractions measured as an experimental:control ratio. The radiation characteristics for Lawrence Radiation Laboratory (LRL) were 250 kV, 5 mA, HVL 1.60 mm Cu; for Massachusetts General Hospital 280 kV, 19 mA, HVL 1.3 mm Cu; for Oak Ridge National Laboratory 250 kV, 15 mA, HVL 0.4 mm Cu, and for Salem, Oregon (the current project) 190 kV and 4.5 mA.

through the view ports provided (Figs. 1 and 2). The X-ray tubes rested in cradles which were now locked in position and the heads could be returned in one location only, if withdrawn for servicing.

The box housing the X-ray tubes was completely lined with 1/16 in. lead reinforced at strategic points as determined by direct radiation survey. The exposure dose rate at gonadal height, 8 ft from the unit, was reduced to 20 mR/hr at maximum operation. The control panel was at the end of 20 ft of cable, and therefore this level of protection was considered adequate in this context. The midbody exposure dose rate to the subject was of the order of 20 mR/hr also. This was achieved by heavier protection of the top and inner faces of the unit. Measurements were made at a point in the middle of the vessel in a place 5 cm below the upper surface (a) in air, and (b) in water. From this data all dose rates throughout the cup could be related to a simple "in air" calibration carried out at the site of the experiment.

In use, the X-ray units were operated until thermal equilibrium was reached. The Victoreen chamber was used to measure the output of each tube head separately to check performance. Calibration of both units operating together was then made in the place, as described below. From this the average dose rate in the water-filled cup and the time for delivery of the desired dose could be computed. The timer was of the Liebel Florsheim type; if the exposure times were less than 1 min the tube currents were reduced and the units recalibrated to minimize timing errors.

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In application, the cup was then 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 pre-determined 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 Checks

One independent check of the X-ray calibrations was made using R-meters by a physicist of 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. E. 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 hr after X-ray. The preserved testicular specimens were analyzed and the results compared to results obtained using equipment at other institutions (Fig. 4).

Mice S. Humans

Data on Irradiation of Subjects

Sixty-seven subjects received acute testicular irradiation in doses between 8 and 600 rad. 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 5 rad/wk for 11 wk. Each dosage given is listed separately in Table I.

Selection of Subjects

The 67 subjects for this study were healthy normal men* between the ages of 25 and 52 yr. 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.

*The men were volunteers who were familiarized with the project and its goals over an extended pre-irradiation observation period to enable the investigators to ensure informed consent. Where applicable, the participant's wife was also required to give written consent. Extensive review of this program was carried out at 3-mo intervals by an approved human consent review board. Three additional reviews were provided by ad hoc committees whose members were selected nationally. Please contact the senior author for further information.

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

Dose grouping for data analysis (rad)	Actual X-ray dose (rad)	Irradiation date	Subject age (yr)
8	8.0	10-12-67	28
	8.0 ^a	10-12-67	27
	8.0	10-12-67	29
	8.0 ^a	10-12-67	27
10	9.4	4-14-65	41
	9.7	2-2-67	43
	10.5	2-17-67	36
15	13.9	11-23-63	30
20	18.2	0-26-65	25
	18.2	2-5-64	26
	18.2	8-5-64	—
	18.2	4-14-65	31
	18.0	10-13-65	36
	20.0	5-6-71	—
	20.0	9-12-70	42
25	23.1 ^a	11-23-63	29
	23.2 ^a	10-13-65	30
50	45.8	0-29-65	31
	45.8 ^a	9-29-65	42
	46.3 ^a	11-23-63	40
	47.0	9-14-63	32
	47.0	9-14-63	52
75	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	—
100	92.6	11-23-63	31
	93.1	12-10-64	24
	93.2	8-22-63	30
	93.2	8-22-63	—
	93.6	3-19-64	31
	93.6	3-19-64	37
	94.0	0-14-63	—
	97.5	2-2-67	46
	98.2	10-13-65	38
	101.0	2-17-66	28

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TABLE I—Continued

Dose grouping for data analysis (rad)	Actual X-ray dose (rad)	Irradiation date	Subject age (yr)
	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	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
230	228.0	10-12-67	41
300	280.5	8-22-63	37
	280.5 ^a	8-22-63	29
400	367.0	9-14-63	40
	375.3	8-5-64	25
	547.5	7-21-64	49
	549.9	2-18-65	41
	554.4	3-14-65	45
	556.4	8-5-64	28
	564.5	4-14-65	—
600	600.0	11-14-68	35
	600.0	3-21-70	41
	600.0 ^a	11-14-68	28
	600.0 ^a	3-21-70	49
	600.0	11-14-68	30
	600.0	1-6-71	34
	600.0	1-9-71	25
	600.0 ^a	5-6-71	31
	600.0	11-14-68	33
	600.0	9-12-70	37
50	Received 11	From	34
TOTAL	dose of	2-18-65 to	
	4.6 rad (avg)	4-29-65	
		Each dose	
		separated	
		by 7 days	

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

Additional routine tests such as blood cytology, urinalysis and various blood chemistry tests were evaluated during the pre- and postirradiation period.

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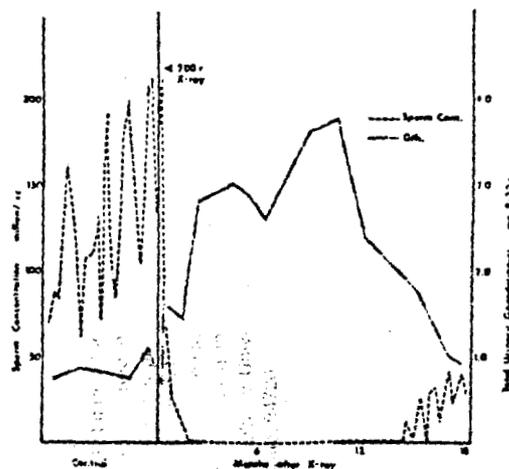


FIG. 5. The response of urinary total gonadotropin levels and sperm concentration to a single dose of 200 rad of X-irradiation to the testes.

Postirradiation Evaluation

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 postirradiation period in order to specifically evaluate sperm and hormonal evaluations (9). After 19 irradiations, biopsies were avoided only during the first 90-day cell depletion period (9) (subject receiving 5 rad/wk tabulated as one irradiation only). All biopsies were prepared for light microscopy (10) and, in the later years of the study, for electron microscopy (11). Germinal cell and Leydig cell quantitation methods were developed (3, 12) 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 (13) 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 (14). Total gonadotropins were bioassayed by the method of Lauson, Heller and Sevringhaus (15). Luteinizing hormone bioassay was conducted using the male hypophysectomized rat method of Greep *et al.* (16). The Steelman-Pohley assay (17) was used for FSH. Estrogens were bioassayed using the method of Lauson, Heller, Golden and Sevringhaus (18). Urinary testosterone and epitestosterone levels were determined using glc (19, 20).

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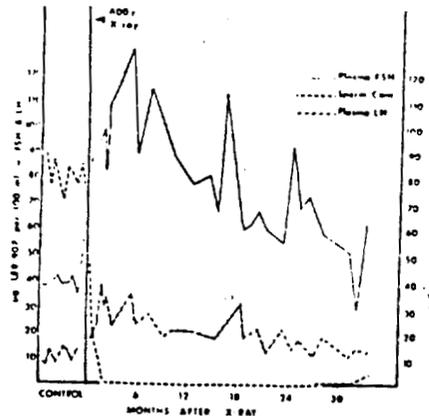


FIG. 6. The response of plasma follicle-stimulating hormone, plasma luteinizing hormone and sperm concentration to a single dose of 600 rad of X-irradiation to the testes.

Plasma testosterone was determined using the method of Murphy (21). Radioimmunoassay of plasma FSH and LH was performed using modifications of methods by Odell et al. (22, 23).

RESULTS

Urinary Gonadotropins (Fig. 5)

In each subject and for each radiation dose level (except 8 rad for which urinary total gonadotropin values were not determined), a distinct rise in urinary total gonadotropins was noted. The rise occurred concomitantly with the first denuding of the germinal epithelium. The rise was sustained until histological recovery began. Subsequent lowering of total gonadotropin levels to normal, paralleled the repopulation of the seminiferous tubules. Urinary LH levels were not changed following irradiation exposure at any time during the course of depletion, quiescent period, or during recovery. Urinary FSH levels paralleled the rise and fall of total gonadotropins. The four subjects receiving 8 rad revealed no change in FSH.

Plasma Gonadotropins (Fig. 6)

Measuring plasma FSH levels confirmed the results obtained from urinary assays. No change was found following 8 rad, a slight increase was seen at 20 rad and highly significant increases were found between 75 and 600 rad. At these dosages plasma FSH levels rose as much as fourfold. Plasma LH levels contrasted sharply with values obtained from urinary assays. Only two dosages (8 and 20 rad) revealed no change in plasma LH following irradiation. At the higher doses (75 and 600 rad) definite LH increases were found. The greatest

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TABLE II
SERTOLI CELL RATIOS (# SERTOLI CELLS/# GERMINAL CELLS) OF BIOPSIES TAKEN FROM ONE SUBJECT BEFORE AND AFTER 100 RAD AND ONE SUBJECT BEFORE AND AFTER 600 RAD OF TESTICULAR X-RAY IRRADIATION*

	Spermatogonia			Spermatocytes				Spermatids			
	Ad	Ap	B	R	L	Z	P	Sa	Sb	Sc	Sd
100 rad											
Control	0.71	0.53	0.28	0.24	0.43	0.06	1.09	1.43	1.29	1.29	1.00
24 hr	0.62	0.44	0.14	0.14	0.33	0.02	1.32	0.72	0.79	0.71	0.74
14 days	0.59	0.06	0.003	0	0.009	0	1.05	0.55	0.59	0.73	0.49
23 days	0.20	0.09	0.04	0.02	0	0	0.27	0.81	1.06	0.75	0.73
49 days	0.27	0.12	0.02	0.03	0.04	0	0.20		0.17		0.32
112 days	0.06	0.02	0	0	0	0	0.03		0.02		0.06
210 days	0.06	0.06	0.005	0	0	0	0.06		0.03		0.02
600 rad											
Control	0.56	0.44	0.29	0.33	0.61	0.01	2.60	1.58	2.20	1.83	1.60
22 hr	0.47	0.24	0.07	0.07	0.74	0.04	2.90	1.88	2.24	2.22	1.66
14 days	0.28	0.23	0.004	0	0.01	0	1.39	0.44	0.99	0.71	0.72
29 days	0.21	0.14	0.01	0	0	0	0.01		1.34		2.37
84 days	0.01	0.002	0	0	0	0	0	0	0	0	0
151 days	0.002	0	0	0	0	0	0	0	0	0	0
252 days	0.006	0	0	0	0	0	0	0	0	0	0
322 days	0.003	0.01	0.02	0.005	0	0	0.05		0.04		0.01
477 days	0.001	0	0	0.006	0	0	0.01	0	0	0	0

* All biopsies were quantitated by one individual by the tubular method (5). Spermatogonia designated Ad (A-dark), Ap (A-pale), and B. Spermatocytes designated R (resting or preleptotene), L (leptotene), Z (zygotene) and P (pachytene). Spermatids designated Sa, Sb, Sc, and Sd, with the Sd being the most mature or differentiated.

increase was after 600 rad where plasma LH levels were double the pre-irradiation values.

Urinary and Plasma Steroids

Urinary estrogen levels were not changed following irradiation. Urinary testosterone levels revealed a minimal but statistically significant lowering following irradiation. Plasma testosterone determination showed no statistical change following irradiation.

Cellular Effects

Spermatogonia were found to be the most radiosensitive cell type with both morphological and quantitative changes at all dose levels (Table II) (except 8 rad where no biopsies were taken). Spermatocytes were overtly damaged at doses of 200-300 rad as shown by their inability to complete maturation division which caused a decrease in resulting spermatid numbers. Spermatocytes were visibly damaged at the 400-600 rad level. Spermatids showed no overt damage,

• Hist
• Recr

however increased. Followed the addition of the sperm (Fig. 5) modern azoospermia.

At the time of the introduction of sperm, the ducts were not yet formed. Preliminary studies in Leydig and Sertoli cells until they were obtained on subjects.

The biopsies precede (Table I) of 200 rad related sperm before sp

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TABLE III
DOSE COMPARISON OF THE INITIATION OF RECOVERY

Dose (rad)	Time of beginning histological recovery (mo) ^a	First sperm in seminal fluid or first increase in sperm numbers (mo) ^b
8		Decrease transient or nonexistent
20	6	6
100	7	7
200	7	11
600	7.5	24

^a Histological recovery initiation calculated by first increase in germinal cell numbers.
^b Recovery initiation by sperm number increase was determined in ejaculated seminal fluid.

however, after 400-600 rad, the resultant spermatozoa were significantly decreased in number signifying covert spermatid damage.

Following testicular depletion through cellular maturation (and the failure of the spermatogonia to supply replacement cells) which takes 46 days, an additional 21 days was required (a total of 67 days following irradiation) for the sperm concentration to drop to azoospermia at all dose levels above 78 rad (Fig. 5). Doses of 78 and 50 rad produced marked oligospermia decreasing sperm concentration from normal to about 2 m/cc. Lower doses produced moderate oligospermia (lowering counts to above 10 m/cc). At 78 rad total azoospermia occurred in all subjects but one.

At the higher doses (400-600 rad) sperm concentration fell precipitously prior to the 46 day period because of the damage to spermatids (Fig. 6). At the intermediate dose levels (200-300 rad) the sperm concentration did not show a decrease prior to the 46 day postirradiation period even though some spermatocytes failed to develop. The mixing of generations of spermatozoa in the ductular system and the natural high weekly variation in sperm concentration may have obscured the diminution.

Preliminary results from subjects at the 600 rad dose level reveal an increase in Leydig cell numbers (counts were corrected for tissue shrinkage by using a Sertoli cell ratio (12), i.e., # Leydig cells/# Sertoli cells). This increase in Leydig cell numbers occurred 90 days after irradiation and remained elevated until the time at which the germinal epithelium began to recover. These results were obtained from four subjects receiving 600 rad and were not determined on subjects receiving the lower doses.

The beginning of recovery as shown by an increase in germinal cell numbers precedes the show of sperm or the increase in sperm numbers in the ejaculates (Table III). At dosages below 100 rad this difference is negligible. At dosages of 200 rad the increase in germinal cells may precede the observation of ejaculated sperm by 4 mo, at 600 rad histological recovery may be initiated 17 mo before sperm are found.

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Complete recovery as shown by a return to pre-irradiation sperm concentrations and germinal cell numbers is within 9-18 mo for doses of 100 rad and below, 30 mo for doses of 200 and 300 rad, and 5 or more years for doses of 400 and 600 rad (only one subject followed to complete recovery at the high dose level).

In the seven subjects that were irradiated a second or third time following complete recovery, the response to the repeated dose was in every way comparable to the initial-dose response.

DISCUSSION

The first problem associated with this project was to deliver as uniform an amount of radiation as possible to all depths of testicular tissue of both testes without exposing the subject to any extraneous radiation. We rejected the conventional X-ray therapy units on the market (in 1963) as unsuitable because of lack of means of uniform coverage of the testes, lack of assurance that uniformity from subject to subject could be established and lack of (or awkward) shielding protection to the subject's body. Therefore, a simple portable box was designed that allowed the scrotum and testes to drop into a plastic box filled with water at scrotal temperature and then be irradiated by two X-ray tubes.

Throughout the time when irradiation was performed (8-22-63 to 5-6-71) the same equipment was used. The irradiations were carried out by a radio-therapist with a technician assisting.

During the early years of this study, urinary gonadotropins and steroids were determined. As plasma methods became available they were substituted for the less accurate bioassay techniques. Generally the results obtained from urinary and plasma analysis agreed. Our explanation for the rise in gonadotropins is that the germinal cells, during their normal course of maturation and development, require gonadotropins. In the absence of germinal cell activity no utilization occurs and consequently excess gonadotropins appear in the plasma and urine. The lowering of gonadotropin values occurs as the germinal cells again become active during the recovery period. A direct comparison between sperm count and gonadotropin curves on any one subject will demonstrate this dependence.

Estrogen and testosterone levels can be used as a measure of Leydig cell function for the human male. The urinary estrogen method was relatively insensitive however, and testosterone determination was later substituted. The fall in urinary testosterone coupled with the rise in LH (plasma) strongly suggest that the radiation caused some interference in Leydig cell function. We suggest that the increase in Leydig cell numbers found at the higher X-ray doses was an effort to compensate for their reduced function.

From the combination of morphological and quantitative data we conclude that spermatogonia are the most radiosensitive cell types and spermatids the most radioresistant cells (Table II). A striking aspect of this data is that cells literally next to each other both in development and spatial placement within the tubule have different radioresistance. For instance, the type B spermatogonia

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HUMAN TESTICULAR IRRADIATION

gonium is the most radiosensitive cell. The cells preceding it (the Ad and Ap) are also radiosensitive. However, the cell arising from the B spermatogonium, the proleptotene spermatocyte, requires tenfold the amount of X-irradiation that the B requires to be damaged similarly. The next major difference in cell radiosensitivity is between the pachytene spermatocyte and the Sa spermatid. Again these cells are adjacent both developmentally and spatially, yet the spermatid requires four times the amount of radiation to be damaged to the same extent. The spermatids are, therefore, 40 times as resistant to radiation damage as are the spermatogonia.

The initiation of recovery was determined using two parameters, sperm reappearance (or number increase) in the ejaculate and the first resurgence of spermatogonia. A large dichotomy was seen between the initiation of testicular recovery versus a sperm count increase. The germinal epithelium began recovery significantly prior to any increase of sperm count. This time difference is probably accentuated by the fact that the germinal cell renewal pattern in man seems to be slightly more radiosensitive than in animals. For example, in mice, at doses of 600 rad and below, spermatogonia tend to repopulate themselves in all tubules before beginning differentiation into spermatocytes and spermatids (24). The response at doses of 800 rad and higher is similar to that of man (25). In man spermatogonia act in an unpredictable manner, producing spermatogonia or spermatocytes at random. A biopsy taken during early recovery has some tubules with spermatogonia, spermatocytes, etc., while an adjacent tubule will be devoid of more than a single spermatogonium. The early differentiation of some spermatogonia (rather than repopulating themselves) caused a further secondary depletion of spermatogonial reserves and greatly prolongs the recovery time. The higher the X-ray dose the more apparent this self-defeating phenomenon.

However, at all doses and after multiple radiation doses, complete recovery does eventually occur.

We concluded that a single exposure of the testis to ionizing radiation at dose levels of 600 rad or below, causes significant disturbance of testicular cytology and hormonal levels associated with cellular processes. These cellular changes and hormonal changes are dose-dependent. More detailed reports of the various parameters are being prepared for subsequent publication.

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REFERENCES

1. C. G. HELLER, G. V. HELLER, and M. J. ROWLEY, Human spermatogenesis: An estimate of the duration of each cell association and of each cell type. III International Congress of Endocrinology, June 30-July 5, 1968, Mexico City, *Excerpta Med. Found. Int. Congr. Ser.* 184, 1012-1018 (1969).

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