# EFFECTS OF X-RAY IRRADIATION ON HUMAN SPERMATOGENESIS

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Direct cell kill and inhibition of mitosis have been suggested as mechanisms to explain the occurrence of absolute sterility following the 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. A total of 209 normal adult non-Catholic inmate volunteers at the Washington State Penitentiary 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 = .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 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 cell kill and mitosis halting mechanisms were operating. Estimates of ED50 of 75r and 27r for cell kill and mitosis halting, respectively, were obtained. The maximum observed sterile period was 501 days with eventual recovery observed in each individual where the follow-up was complete. Thus man appears to be highly radiosensitive in regard to temporary sterility but quite radioresistant in regard to permanent sterility.

Various mechanisms suggested by animal experiments have been postulated to explain the phenomenon of absolute sterility or azoospermia resulting from irradiation of the testes.

Oakberg (ref. 1) attributes the observed depletion or total absence of germ cells in the epithelium of the seminiferous tubules to be due to direct cell kill. Under this hypothesis the length of the pre-sterile period or time to first azoospermia would be determined by the time required for the resistant cells to complete development and reach the ejaculate. The duration of azoospermia or length of the sterile period is considered to be made up of two components. The duration of the first component is equivalent in length to the age of the youngest cohort of germ cells that was not totally decimated. The second component of the sterile period is the time required for the surviving stem cells to multiply sufficiently to reach a critical density or cluster size necessary for differentiation to take place.

Jones (ref. 2) postulates that an inhibition of mitosis may be the mechanism responsible for the sterile period. Under this hypothesis the time to first azoospermia would be equivalent to the total maturation time plus the time post-irradiation required for mitosis to be halted. The duration of the sterile period is identical to the time required for the mitotic mechanism to repair itself.

The existence and dose dependency of the postulated mechanisms with respect to human beings is of obvious scientific interest and growing medicolegal importance as human exposure to radiation increases. The variability in spermatogenic kinetics observed among various species in regard to their gonadal response to dfiferent levels of radiation (refs. 3 to 5) renders any human sensitivity estimates based on animal data highly questionable.

## NORMAL HUMAN SPERMATOGENIC KINETICS

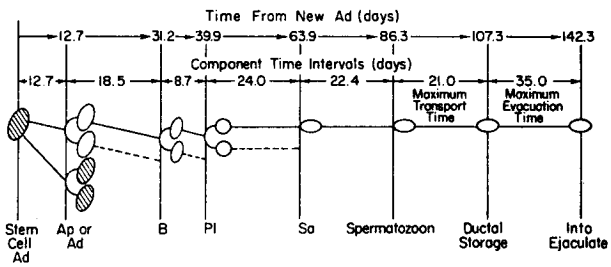
In recent years a series of experiments were performed that allow one to estimate the time required for a human germ stem cell to mature and be passed into the ejaculate.

Clermont (ref. 6), Heller and Clermont (ref. 7) have devised a highly explicit model for the mode of development and timing of human spermatogenesis. The mode of development portion of the model is based on the observed ratios of germ cell types in various stages of maturation counted in biopsy specimens. The timing relationships were determined by using radioautographs of testicular sections following intratesticular injections of $H^3$-thymidine. The maximum transport time of the mature spermatozoa from their release from the Sertoli cell cytoplasm into the ejaculate has been determined by Rowley et. al. (ref. 8) to be no more than twenty-one days. Freund and Davis (ref. 9) observed a thousand fold depletion of sperm count at the fifth ejaculation post vasectomy. Thus the time required to evacuate ductal storage of spermatozoa is virtually equivalent to the time to the fifth ejaculation.

The minimum time required for the mature spermatozoa to reach the ejaculate under certain conditions is considered to be less than one day.

A model for the maximum time required for a maturating human germ stem cell to reach the ejaculate based on the preceding considerations is presented in a schematic manner in figure 1. This model will be utilized to test the plausibility of the various suggested disrupting mechanisms in regard to human spermatogenesis.

FIGURE 1 MODEL FOR MODE OF DEVELOPMENT AND TIMING OF HUMAN SPERMATOGENESIS




Abbreviations: Ad, A dark spermatogonia; Ap, A pale spermatogonia; B, B spermatogonia; Pl, Preleptotene primary spermatocyte; Sa, Sa spermatid.

## HUMAN EXPERIMENT OF THE EFFECT OF X-RAYS

In order to obtain a reliable estimate of the existence and dose dependency of the postulated spermatogenesis disrupting mechanisms for man, a controlled experiment was undertaken in 1963. This study utilized a group of 209 healthy non-Chatholic inmate volunteers at the Washington State Penitentiary in Walla Walla. Each inmate selected for the study had to express a desire for a vasectomy. This was to be performed at the completion of his involvement in the study. The inmate and, if married, his wife both signed a written consent form authorizing all experimental procedures.

Extensive pre-irradiation sperm samples were obtained by masturbation for each inmate and sperm density estimates of ejaculate made to demonstrate normal spermatogenesis. The testes of sixty-four of the men received a mid-organ dose of X-rays ranging from 7.5 to 400.r. (f factor = .95) administered acutely by a Maximar 250 Kv unit. The remaining 145 men served as various types of controls. In addition, in order to obtain direct information on testicular morphological changes, periodic unilateral testicular biopsies were performed on a number of individuals in both the irradiated group and non-irradiated group. Weekly sperm samples were collected post-irradiation and sperm density estimates made even though the end point of central interest was azoospermia.

The number of inmates in each biopsy-radiation group and the number of these that were observed to have achieved azoospermia by 142 and by 210 days is shown in table 1. The duration in days of the pre-sterile period and the sterile period for each inmate in his biopsy-radiation group that became azoospermic is given in table 2. The question marks (?) indicate that the individual was lost for observational purposes while still azoospermic.

*Table 1*

DATA USED TO ESTIMATE PARAMETERS IN POSTULATED MODELS

| X-Ray (r) | Number of Biopsies | Fraction Sterile by 142 Days | Fraction Sterile by 210 Days |
|---|---|---|---|
| 400 | 0 | 2/2 | 2/2 |
| | 1 | 1/1 | 1/1 |
| | 2 | 2/2 | 2/2 |
| 100 | 0 | 5/7 | 7/7 |
| | 1 | 7/9 | 9/9 |
| | 3 | 2/2 | 2/2 |
| 50 | 0 | 1/8 | 5/8 |
| 7.5 | 0 | 0/5 | 0/5 |
| 0 | 0 | 0/96 | 0/96 |
| | 1 | 0/29 | 0/29 |
| | 2 | 1/18 | 1/18 |
| | 3 | 0/1 | 0/1 |

*Table 2*

TIME SEQUENCE OF AZOOSPERMIA AS A FUNCTION OF RADIATION AND BIOPSIES

| X-Ray (r) | Number of Biopsies | Pre-Sterile Period in Days | Sterile Period in Days |
|---|---|---|---|
| 0 | 2 | 38 | ?>509 |
| 30 | 0 | 95 | 182 |
| | 0 | 194 | 7 |
| 50 | 0 | 115 | 70 |
| | 0 | 160 | 91 |
| | 0 | 171 | 84 |
| | 0 | 195 | 21 |
| | 0 | 202 | 49 |
| 100 | 0 | 88 | 209 |
| | 0 | 107 | 91 |
| | 0 | 129 | 112 |
| | 0 | 141 | 112 |
| | 0 | 141 | 112 |
| | 0 | 152 | 61 |
| | 0 | 189 | 112 |
| 100 | 1 | 65 | 108 |
| | 1 | 100 | 421 |
| | 1 | 109 | ?>42 |
| | 1 | 111 | ?>7 |
| | 1 | 113 | 112 |
| | 1 | 114 | 232 |
| | 1 | 124 | ?>152 |
| | 1 | 201 | ?>21 |
| | 1 | 209 | 62 |
| 100 | 2 | 145 | 327 |
| 100 | 3 | 127 | 291 |
| | 3 | 127 | 501 |
| 400 | 0 | 76 | 147 |
| | 0 | 98 | 196 |
| 400 | 1 | 115 | 329 |
| 400 | 2 | 113 | 338 |
| | 2 | 116 | 342 |

The 142 day time period was selected since it represents the maximum pre-sterile period possible if the mechanism causing sterility is complete cohort decimation. This estimate is based on the observation that if an inmate masturbated only at his prescribed collection time evacuation would be accomplished in 7 x 5 = 35 days. Thus the maximum pre-sterile period is 35 + 21 + 86 = 142 days. The 210 day time period was selected since no new occurrences of azoospermia were observed after that time.

## MODELS FOR THE EFFECTS OF X-RAYS

From figure 1 we note that a pre-sterile period of less than 86 days can only be explained in terms of the postulated mechanisms by assuming death of a cohort of differentiating germ cells. For two inmates azoospermia occurred at 65 and 76 days confirming the existence of that mechanism. If the pre-sterile period was greater than 142 days then complete cohort decimation did not occur. A lower limit on the dose dependency of the cohort decimation effect can be determined by assuming that if azoospermia occurs by 142 days the effect occurred. Thus the observed fraction of inmates becoming azoospermic by 142 days shown in table 1 can be utilized to estimate the $ED_{50}$ of the effect.

A model relating the probability of total cohort decimation to the dose of X-rays can be derived under the following assumptions:

i) The probability of the most sensitive cell type being killed given a dose of X-rays of size x follows a one-hit-curve of the form

$$P = (1 - e^{-\theta x}).$$

ii) The cohort size of the most sensitive type of germ cell of an individual is a random variable N which has a poisson distribution with parameter $\lambda$.

iii) A person is azoospermic if all N of his most sensitive type of cell are killed.

Under these assumptions the probability of total cohort decimation is

$$P(x) = \sum_{N=0}^{\infty} (1 - e^{-\theta x})^N \, e^{-\lambda} \frac{\lambda^N}{N!} = e^{-\lambda e^{-\theta x}}$$

and the dose of radiation that results in 50% of the population becoming azoospermic due to cohort decimation is

$$1/2 = e^{-\lambda e^{-\theta x_{50}}}$$

or

$$x_{50} = ED_{50} = [-\ln(\ln 2) + \ln \lambda]/\theta$$

The dose required ($LD_{50}$) to kill 50% of the most sensitive type of germ cell can also be estimated from this model. It follows directly from the assumption of a one hit curve for cell kill that

$$1/2 = 1 - e^{-\theta x_{50}}$$

or

$$x_{50} = LD_{50} = \ln 2/\theta$$

The parameters of the model ($\lambda, \theta$) were estimated from the observed data by the maximum likelihood method (ref. 10), adjusting for the probability that a testicular biopsy could also independently couse azoospermia by 142 days. The estimate of $ED_{50}$ was found to be 74.6r with a 95% confidence interval obtained by using Fieller's Theorem (ref. 11) of 57.2 to 103.6r. In addition, it is of interest to note that this rather indirect method of estimating the $LD_{50}$ of cell kill gives an estimate of 23.2r which is in remarkable agreement with Oakberg's estimate (ref. 12) of an $LD_{50}$ of between 20 and 24 rad of X-ray for spermatogonia.

The longest observed pre-sterile period was 209 days with 10 out of the 32 inmates who became azoospermic having a pre-sterile period greater than 142 days. The maximum observed sterile period was 501 days where a maximum of 142 - 127 = 15 days could be explained by cohort decimation. To explain these results by the cell kill hypothesis several additional assumptions are needed. We need to assume that some stem cells are capable of functioning normally for at least 209 - 142 = 67 days post-irradiation and then die. For one individual we must assume that the total time where stem colony growth took place if 501 - 15 = 486 days and thus the number of doublings was 486/12.8 = 38 and the total restored stem cell population for an individual tubule is at least $2^{38}$ before differentiation took place. Neither of these assumptions appear to be very tenable.

Oakberg (ref. 13) suggests upon viewing similar results of length of the sterile period for humans based on a preliminary report by Heller (ref. 14) that differentiation of the stem cells produces non-viable daughter cells causing the stem cell population to build up slowly thus prolonging the sterile period. However, on inspection of our testicular biopsies (ref. 15) we observe a period of very low stem cell density followed by a rapid doubling of stem cells in line with the 12.8 day doubling time, which in turn is followed by the differentiation of the stem cells and eventual recovery. In the light of these results it appears reasonable to assume that mitotic division is also halted in some manner by the radiation.

If we assume that individuals have a normal distribution of log dose tolerances with regard to some mitotic division mechanism, then the probability of azoospermia given a log dose of radiation of size x is to a very close approximation

$$P(x) = \frac{1}{1 + e^{-(\alpha+\beta x)}}$$

and

$$x_{50} = ED_{50} = e^{-\alpha/\beta}$$

If we assume that a critical volume in the testes exists that has an overriding control over the mitotic division mechanism and is damaged with one hit, or if we assume that tolerances to dose have the exponential distribution, then the

$$P(x) = 1 - e^{-\theta x}$$

and

$$x_{50} = ED_{50} = \ln 2/\theta.$$

Estimates of the parameters ($\alpha$, $\beta$) or ($\theta$), for these models was obtained by maximum likelihood estimation adjusting for the probability of a biopsy causing sterility, using as data the fractions of individuals who became azoospermic by 210 days. Estimates of $ED_{50}$ were 36.3r with a 95% confidence interval of 19.8 to 50.3r for the normal distribution of log dose tolerance model and 27.2r with a 95% confidence interval of 18.8 to 48.8r for the one hit model. The variation in the confidence intervals for these two models are minimal while the differences in the $ED_{50}$ estimates are primarily due to different dose scales. A summary of the $ED_{50}$ results is shown in table 3.

Simulation experiments were run on these models to verify that the asymptotic approximations used to obtain the confidence intervals were valid for the sample sizes used in the experiment. In all cases a close agreement was obtained between the observed and expected results which virtually assures the statistical validity of the stated intervals.

## CONCLUSIONS

In men the occurrence and length of azoospermia or complete sterility following X-ray irradiation is made up of a number of components. It appears reasonable to assume that complete cohort decimation of some germ cell type does take place following exposure to sufficiently large doses. However, doses that are not large enough to cause total cohort decimation are still able to stop spermatogenesis by halting mitosis of the surviving stem cells for extended periods of time needed for repair. The repaired stem cells multiply until a critical cluster size is reached after which differentiation occurs followed by the reappearance of sperm in the ejaculate.

Man also appears far more sensitive to radiation than the rodent who needs almost ten times the dose to become azoospermic. The time needed for man to recover is also much longer than would be expected from animal experiments, ranging up to a year and a half after exposure. However, the evidence does point to eventual recovery even for doses of 400r.

In short, man is very radiosensitive in regard to temporary sterility periods as long as one and a half years but very radioresistant to complete sterility.


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*Table 3*

$ED_{50}$ ESTIMATES FOR POSTULATED MODELS AND ITS 95% CONFIDENCE LEVEL

| Model P(x) | Days Post Irradiation | Lower Limit | $ED_{50}$r. | Upper Limit |
|---|---|---|---|---|
| $e^{-\lambda e^{-\theta x}}$ | 142 | 57.2 | 74.6 | 103.6 |
| $\frac{1}{1+e^{-(\alpha+\beta \ln x)}}$ | 210 | 19.8 | 36.3 | 50.3 |
| $1-e^{-\theta x}$ | 210 | 18.8 | 27.2 | 48.8 |