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COMPREHENSIVE PROGRESS REPORT OF AEC  
CONTRACT No. AT(45-1)-1781 COVERING  
1963-1969

April 1969

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FOLDER HUMAN IRRADIATION

1001906

RESEARCH PROJECT

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

Comprehensive Progress Report of AEC Contract  
#AT(45-1)-1781 Covering 1963-1969

Prepared April 14, 1969

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INTRODUCTION

1969 marks the completion of our sixth operational year for this research project. It is noteworthy that we have made a major advance in one of our troublesome areas, namely; the statistical method for analyzing the data concerned with dose-related changes in spermatogenesis. A working model has been established (see discussion of sperm concentration changes).

1. Definition of the x-ray induced sperm count "decreases" in terms of rad dosage.
2. Description of the testicular histologic changes during the damage and repair phases following x-ray irradiation.
3. Determined the relationship between decreased spermatogenesis and increased urinary Follicle-Stimulating Hormone (FSH) titers following x-ray irradiation.
4. Uncovered evidence that suggests alterations in Leydig-cell function do occur following irradiation.
5. Established methods for measuring serum luteinizing hormone (LH) and serum FSH titers by radioimmunoassay.
6. Essentially completed a sensitive, accurate method for determining serum testosterone levels by a protein-binding and displacement technique.
7. Obtained preliminary data which supports the contention that a testicular biopsy procedure or exposure to x-ray irradiation may initiate an immunologic response in the germinal epithelium.
8. Completed the mannikin dosimetry studies with the neutron generator.
9. Initiated studies to examine the possible protective influence of testosterone administration prior to x-ray irradiation.

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## DETAILS OF PROGRESS REPORT

I. Status of Inmate Volunteers: Since the inception of our project the procedures used for obtaining volunteers and working with these men have worked well and no major problems have arisen. The following summary lists the numbers of research volunteers and their respective categories:

- A. Total inmates accepted for project (165)
- B. Total men irradiated (60)
- C. Number of inmates dropped from program because of personal reasons or non-related medical problems, not irradiated (41), irradiated (2)
- D. Number of volunteers who have been released from the penitentiary (81)
- E. Number of volunteers who have had a bilateral vasectomy prior to release (40)
- F. Number of irradiated volunteers who did not have a vasectomy; declined and has been released from the penitentiary (1); mutual agreement not to have vasectomy, post-myocardial infarction, currently serving a life sentence (1)

## II. Seminal Fluid Examination

A. Introduction: Seminal fluid sperm concentration represents the critical end-point we have selected to assess changes in sperm production following irradiation. Alternative parameters were examined to determine whether or not they might be more reproducible or more sensitive indicators. These included motility, supravital staining for live/dead ratio measurements and morphology. Dr. Wm. Clarke's group at Hanford were instrumental in this part of our investigation. The results of these studies indicated that motility estimates were not a more sensitive indicator. Furthermore, these estimates were too subjective for consistent reliability. Changes in live/dead ratio paralleled our sperm concentration results and thus did not afford any advantage over the latter parameter.

This year Dr. Thorslund, who joined our project team as a part-time biostatistician, examined our past methods for determining sperm count changes following irradiation and recommended that a new approach be used. The basis for this

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recommendation lies in a study which evaluated the nature of sperm count variation (see section B). Various mathematical models were considered to deal with this problem. The details of this deliberation as well as the model which was used to derive the proposed dose response curve, are given in sections C and D.

- B. The nature of sperm-count variation. It is well recognized that quantitative measurements of the number or density of sperm in separate ejaculates from a given individual are usually different. Thus the measured sperm count is a variable. In any attempt to detect a change in sperm concentration which is due to some physical manipulation, the knowledge of the extent and pattern in this variability is of critical importance. This becomes especially relevant if the detection of possible changes is to be determined by the means of some statistical test.

Any statistical test makes very explicit assumptions about the nature of variability. It follows then that if these assumptions are not conformed to in a given situation then the conclusions reached can be erroneous. Several common assumptions upon which many statistical tests are based include: normality, homogeneity of variance and independence of observations. Before we could finalize our method for analyzing the sperm count data it was necessary to examine the validity of each of these assumptions as they pertained to our situation.

1. Normality

Under the assumption of normality the probability distribution of the variable x sperm count is:

$$(1.1) \quad f(x) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-1/2 \left(\frac{x - \mu}{\sigma}\right)^2}$$

where  $\mu$ ,  $\sigma^2$  are the mean and variance of the sperm count, respectively. The function  $f(x)$  describes the familiar symmetrical bell-shaped curve.

To test the assumption of normality, five individuals who had an excess of fifty separate control sperm counts were selected. The mean and variance of each individual's sperm count were computed. Using the estimated means and variances and the assumption of normality, sperm-count intervals, all of which had an equal probability of a sperm count falling within them, were obtained. The number of intervals obtained was the largest integer k such that  $k \leq n/5$ , where n is the number of control sperm count of a given individual. This insured that we would have the maximum

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number of intervals under the restriction that the expected number of observations in each interval was five or more. The observed number of sperm counts falling into the intervals was then tabulated. A  $\chi^2$  goodness of fit test was run on the resulting data, where  $\chi^2$  has k-3 degrees of freedom. The null hypothesis of the observations coming from a normal distribution was rejected for four of the five individuals at the .05 level. The reason for this was primarily due to an excess of observed observations in the lower intervals. This implies that the true sperm-count probability distribution is probably skewed toward smaller sperm counts.

If the object of an experiment is to detect a difference between means, then it is of little practical consequence if the assumption of normality is adhered to. However, if a quality-control-chart approach that assumes normality is utilized, then the lack of normality could lead to several errors. Generally, the quality-control approach signifies the establishment of a base-line or mean. If subsequent observations are more than a specified number of standard deviation units away from the base-line, then the process is said to be out of control. The number of standard deviation units chosen is determined by the proportion of the time or  $\alpha$  one is willing to say the process is out of control when, in fact, it is not. If sperm-count data are in fact skewed towards the smaller sperm-count values, as our data indicate, then we would be stating that the process is out of control when, in fact, it is not a proportion of the time that can be considerably greater than  $\alpha$ . In other words, we will be making a type I error greater than the claimed value of  $\alpha$ . This type of situation can be depicted visually as is shown in Fig. 1.

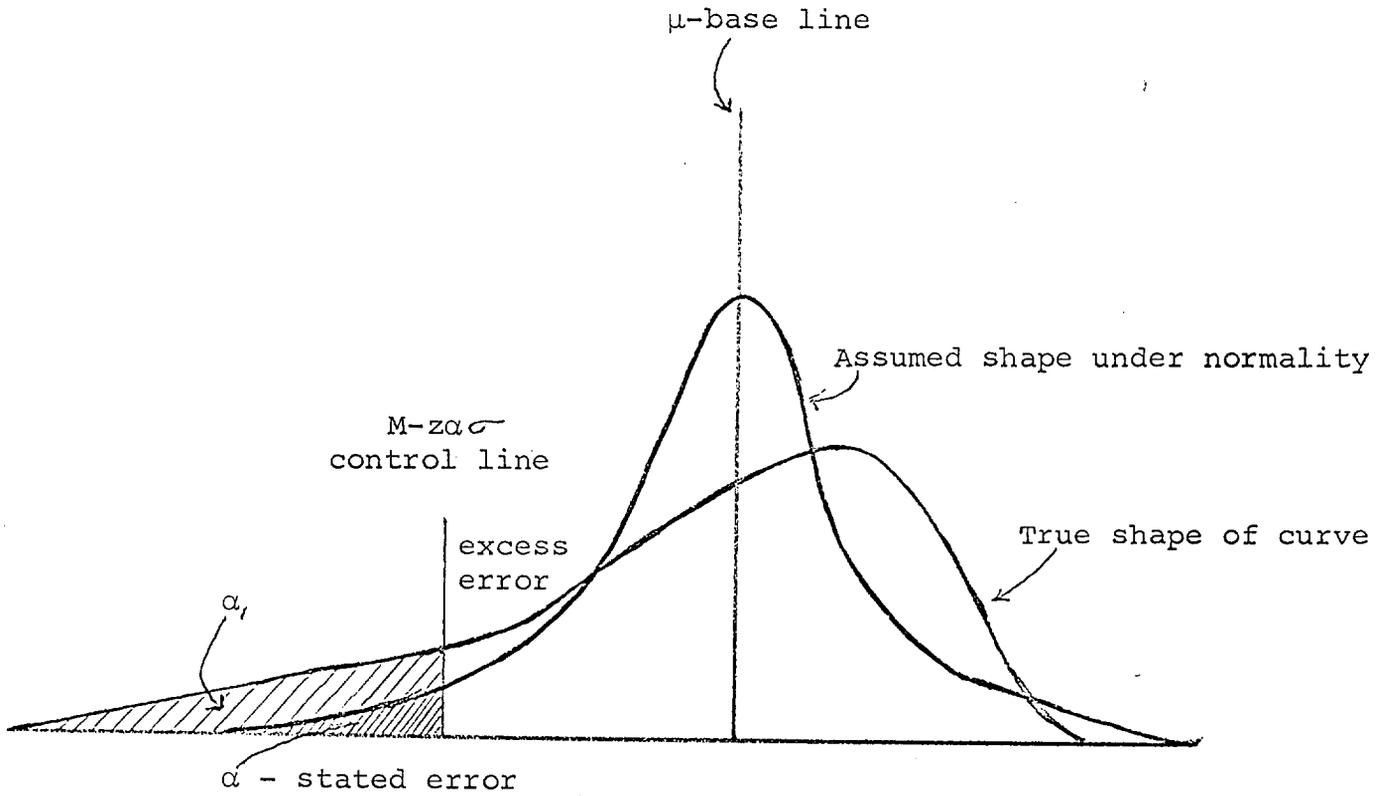
In addition, examination of data from several individuals not included in the present study revealed that the control line, with its standard deviation units, be set at values less than zero. That is using the assumption of normality. This would make it impossible to say that the sperm counts for these individuals were ever out of control, regardless of how low the post irradiation sperm counts became.

Therefore, we can conclude that a quality-control approach which assumes normality cannot be used for our sperm-count data.

## 2. Homogeneity of Variance

The existence of equal variances for all the populations to be compared is an assumption that is made for most parametric and non-parametric statistical tests. Looking

Fig. 1. Hypothetical Relationship Between Normal and True Sperm-Count Probability Distributions



$\alpha + \alpha_1 = \text{true error}$

$\alpha = \text{stated error}$

at our control sperm-count data, it appeared that the variability in sperm counts was directly proportional to the mean sperm count.

In order to test this hypothesis, the following experiment was performed. For 78 individuals who had eight or more control sperm counts, the sample mean and variance were computed and plotted against each other on a graph. The graph indicated that a quadratic-type relationship of the form  $S^2 = \alpha + \beta \bar{x}^2$  might be used to define the relationship between the mean and variance. As a result, the parameters  $\alpha, \beta$  were estimated using the method of least square and the relationship,  $S^2 = a + b \bar{x}^2$ , thus obtained. Next, the null hypothesis,  $\alpha = 0$ , was tested and accepted at the .05 level. This implied that the standard deviation was directly proportional to the mean. This allows us to obtain equality of variances through the use of the variance stabilizing transformation  $\ln x$ .

Thus, in any statistical test used to investigate differences in sperm-count levels, it is concluded that the test should not run on the observed data  $x$  but on the transformed values  $\ln x$ . If the possibility of  $x = 0$  exists, then the transformation could be altered to  $\ln(x + 1)$ .

3. Independence of Observations

Most statistical tests are based on the explicit assumption that if the mean is known then the level of one observation gives you no new information about the level of the next observation. However, if true in our situation, knowing an individual's sperm count one week would give us no more information about what would be next week than just knowing the individual mean sperm count.

To test this assumption eighteen individuals who had sixteen or more weeks of consecutive sperm counts were tested to see if their auto-correlation function over a six-week period was different from zero. Since the sample sizes were relatively small, the exact test had to be used. Because this test requires extensive calculations, a computer program was devised to facilitate the work. The results are shown in Table 1:

	lag time t (weeks between observations)					
	1	2	3	4	5	6
Number significant						
at .05 level	12	7	4	3	2	1
out of 18						

Table 1

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These results indicate that there is some trend based on time for sperm counts of the same individual. This led us to conclude that we had to take maximum care in interpreting sperm-count change after a treatment since some changes could be due merely to a general change over time that would have occurred regardless of irradiation.

In summary, analysis of our control data clearly demonstrates that sperm-count data disobey those conditions a statistician would like to impose upon them. As a result, any difference between sperm count groups cannot be based upon "standard" statistical tests but have to be based upon radical differences. For example, if all the observations in the control group are greater than all the observations in the test group or post-irradiation period then we might conjecture that the sperm counts were reduced.

- C. Mathematical Models. Our ultimate goal is to develop a realistic mathematical model which bears on the mode of action that radiation exerts on human sperm production. Such a model would utilize sperm count data, urinary and/or serum FSH titers and quantitative studies of the germinal epithelium. At the present time a viable method for obtaining histologic quantitation needs to be developed before such a model can be derived and tested.

In the meantime there are several models which have been shown to be useful in predicting all or none effects of radiation. These were investigated to determine their applicability with respect to our data.

1. The Multivariate One-Hit Curve

This model assumes that any one of  $m$  environmental factors acting independently of each other could produce the effect, if the factor destroys the "control volume" of the target organism. This may be written in mathematical notations as:

$$(2.1) P_j = 1 - e^{-(\beta_1 X_{1j} + \beta_2 X_{2j} + \dots + \beta_m X_{mj})}$$

Where the  $X_{ij}$ ,  $i = 1, 2, \dots, m$  are the environmental factors for the  $j$ th individual,  $P_j$  is the probability of the effect, and  $\beta_1, \beta_2, \dots, \beta_m$  are the unknown parameters to be estimated.

## 2. Multivariate Logistic Curve

In this model it is assumed that individuals have a normally distributed tolerance to a sum of environmental factors. Using the notation of the previous model this can be written as

$$(2.2) P_j = \frac{1}{1 + e^{-(\beta_1 X_{1j} + \beta_2 X_{2j} + \dots + \beta_m X_{mj})}}$$

In both instances the equations for estimating the parameters ( $\beta_1, \beta_2, \dots, \beta_m$ ) were obtained by the method of maximum likelihood. The solution is dependent upon solving a set of  $m$  simultaneous non-linear equations, which could take years to complete by hand calculation if  $m$  is greater than one. As a result it was necessary to develop a computer program to obtain the necessary solutions.

The estimates of the parameters can then be utilized to obtain estimates of new parameters such as ED/50, and the maximum likelihood theory can be employed to obtain confidence intervals for these estimates.

There is another facet to the handling of our data that requires consideration; that is the proposed procedure is based upon a mathematical theory which assumes that our sample sizes are approaching infinite size. Therefore, the validity of our results for the small sample sizes which we are dealing with need to be evaluated.

By the use of large scale computers we are provided with an empirical method to check the results obtained by asymptotic theory. This method is called simulation or Monte Carlo techniques. For example, a random sample assuming a specific form and value of parameters of the mathematical model to be investigated is generated for the fixed experimental design which will be used. Then the calculations are performed with these data and the estimates obtained. This process is repeated many times. The mean estimates and variances which are obtained are then compared to the mean estimates and variances calculated from our actual parameters. If the actual and contrived mean estimates are comparable then we can be confident that our theory is applicable for our relatively small sample sizes.

D. Results of Our Sperm-Count Analysis

1. Introduction. The ultimate objective in obtaining a dose-response relationship between radiation and its effect upon human sperm production is to assess the magnitude of danger to male fertility which known amounts of radiation present.

Using a dose-response curve, this type of information may be summarized in the form of ED/P/t estimates, where the symbol ED/P/t is the usual notation for the dose of radiation that will cause the investigated effect to be present in a proportion P of the population by post-irradiation time t.

In obtaining such estimates, two decisions must be reached; the response metameter or biological end-point to be used and the mathematical form of the dose-response curve to be used.

2. Selection of the End-Point. In the present situation, as a minimal requirement, any selected end-point should conform to two very general conditions. An end-point should be affected only by radiation and additional factors that can be adjusted for, and it should have biological significance in its own right.

An end-point that has been used previously, which clearly violates these criteria, is the statistically significant change in sperm count. The probability of detecting a change in sperm counts by statistical methods is not only due to actual change in sperm production but also to the experimental design used and to the statistical test employed. Thus, a lower estimate of an ED/P/t could be obtained simply by increasing the number of control sperm samples or by changing from a non-parametric to an appropriate parametric statistical test, factors that have no bearing on radiation exposure. In other words, what is being measured is not the change in sperm count but the power of the statistical test to say that there is a change in sperm count.

We have chosen to use the end-points less than 5 million sperm/cc. of ejaculate and azoospermia. The former is an expedient and useful definition of clinical sterility and the latter, an absolute definition of sterility. These end-points have the advantage of being measureable with very little error and are highly unlikely to be produced by any other factor than radiation or some other known physical manipulation.

3. Selection of the Mathematical Model. Ideally, a mathematical model should be derived from a consideration of the biological factors of the problem being investigated. It should be as simple as possible and predict the data obtained within the limits of the

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statistical variability associated with the process. At the present time there is insufficient information available about the mode of action of radiation on human sperm production to facilitate the development of a highly realistic mathematical model. However, a model that has been proven to have considerable utility in somewhat analogous situations could be extended and employed in our problem.

Lea (1946) first proposed the one-particle or one-hit dose-response curve in reference to radiation data. This model assumes that the effect of radiation is due to a single ionization occurring in the "control volume" of a biological organism. Under this assumption it can be shown that the probability of the effect  $P_i$  occurring given a radiation dose  $X_i$

$$(1) \quad P_i = 1 - e^{-\beta X_i}$$

where  $\beta$  is an unknown parameter to be estimated. This model can be extended to account for more than one factor influencing the probability of the effect occurring, if it is valid to make the assumption that the factors are acting independently of each other. In this case the model may be written as

$$(2) \quad P_i = 1 - e^{-(\beta_1 X_{1i} + \beta_2 X_{2i} + \dots + \beta_m X_{mi})}$$

where there are  $m$  unknown parameters and  $m$  known environmental factors or constants. For example,  $X_{1i}$  might be a 1 if the  $i$ th individual had a biopsy post-irradiation and a 0 if he did not.  $X_{2i}$  is the dose of x-rays given the  $i$ th individual and  $X_{3i}$  is the dose of neutrons given the  $i$ th individual, etc.

The parameters,  $\beta_1, \beta_2, \dots, \beta_m$ , can be estimated by the method of maximum likelihood, and these estimates, in turn, can be used to estimate other parameters of interest. For example, in the previous given situation,  $\beta_2/\beta_3$  would be an estimate of the relative biological effect of x-rays to neutrons and  $\ln 2/\beta_2$  would be the ED/50/t of x-rays.

4. Results. The data obtained are shown in tables 2 and 3. The numerators are the number of individuals, where the effect occurred by time  $t$  and the denominators are the number irradiated or sample size.

The change in sample size over time is due to either the subject voluntarily removing himself from the study or the data being taken for analysis before the individual had reached that particular post-irradiation time. The time was not extended beyond

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Table 2. The number with the observed effect  $< 5$  M/cc. divided by the number exposed at different radiation doses and times post-irradiation

X-ray Dose (r)	Time in Days Post-Irradiation				
	90	120	150	180	210
400.0	3/3	3/3	3/3	3/3	3/3
100.0	3/4	3/4	4/4	4/4	4/4
50.0	5/8	6/8	6/8	7/8	6/6
30.0	0/3	2/3	1/2	1/2	2/2
15.0	0/7	0/7	0/7	0/6	1/3
7.5	0/5	0/5	0/5	0/5	0/3

Table 3. The number with the observed effect azoospermia divided by the number exposed at different radiation doses and times post-irradiation.

X-ray Dose (r)	Times in Days Post-Irradiation				
	90	120	150	180	210
400.0	1/3	3/3	3/3	3/3	3/3
100.0	1/4	2/4	2/4	3/4	4/4
50.0	0/8	1/8	1/8	3/8	4/6
30.0	0/3	0/3	0/2	0/2	1/2
15.0	0/7	0/7	0/7	0/6	0/3
7.5	0/5	0/5	0/5	0/5	0/3

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210 days since no new effects were observed beyond that point.

The first model used is defined by equation (1) where  $X_i$  is x-ray dose in rads. It has been shown that much greater precision is obtained by estimating ED/50/t rather than some other percentile point. Under the present model the estimator for ED/50/t is  $\ln 2/\beta$  which has an approximate variance  $V(2)\ln^2 2/\beta^4$ , where  $V(\beta)$  is obtained from the inverse of the information matrix of the maximum likelihood solution. Using this relationship an approximate  $(1-\alpha)$  percent confidence interval for ED/50/t of the form

$$(3) \quad \ln 2/\beta \pm Z_\alpha \ln 2 \sqrt{V(\beta)}/\beta^2$$

may be obtained, where  $Z_\alpha$  is the  $\alpha$  percentile point on the standard normal curve. This is only an approximate result, the validity of which is being investigated by a simulation experiment.

The estimates of ED/50/t and their confidence intervals are given for the end-points less than 5 million/c.c. and azoospermia in Tables 4 and 5 respectively. The validity of these estimates are dependent upon the ability of the mathematical model to predict accurately the observed data.

The model does appear to give adequate predictions for all cases. However, since the data between times and end-points are highly correlated we will only test the limiting case of azoospermia at 210 days post-irradiation. Fig. 2 shows the comparison between the observed data and the theoretical dose-response curve for this limiting case. These data can also be represented in the form of a table which compares the "observed affected" with the "expected affected" which are computed based upon the assumption that the mathematical model holds. Such a comparison is shown in Table 6. A  $\chi^2$  goodness of fit test yields a value  $\chi^2 = 2.34$  with 5 d.f. which has a p value of .8 associated with it. This does not imply that a one-hit type mechanics situation is an accurate description of the radiation-human-sperm-production interaction. It only indicates that at our present state of knowledge, we do not have enough information to say it is not so. Thus, the use of any more complicated model would be of limited utility.

It should be pointed out that all of the ED/50/t estimates are highly correlated since they are obtained from various subsets of the same sample of individuals. Thus, any description of the time-course of ED/50 is highly tenuous. However, each estimate is still the best one available from the limited data.

Finally we are aware of the fact that for our results to be applicable to man in general the assumption must hold that our prison volunteers are a representative sample of man with respect to sperm production. There are no data to suggest that this assumption is false.

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Table 4. ED/50 estimates and .95 confidence interval for the end-point < 5M/cc. at various times post-irradiation.

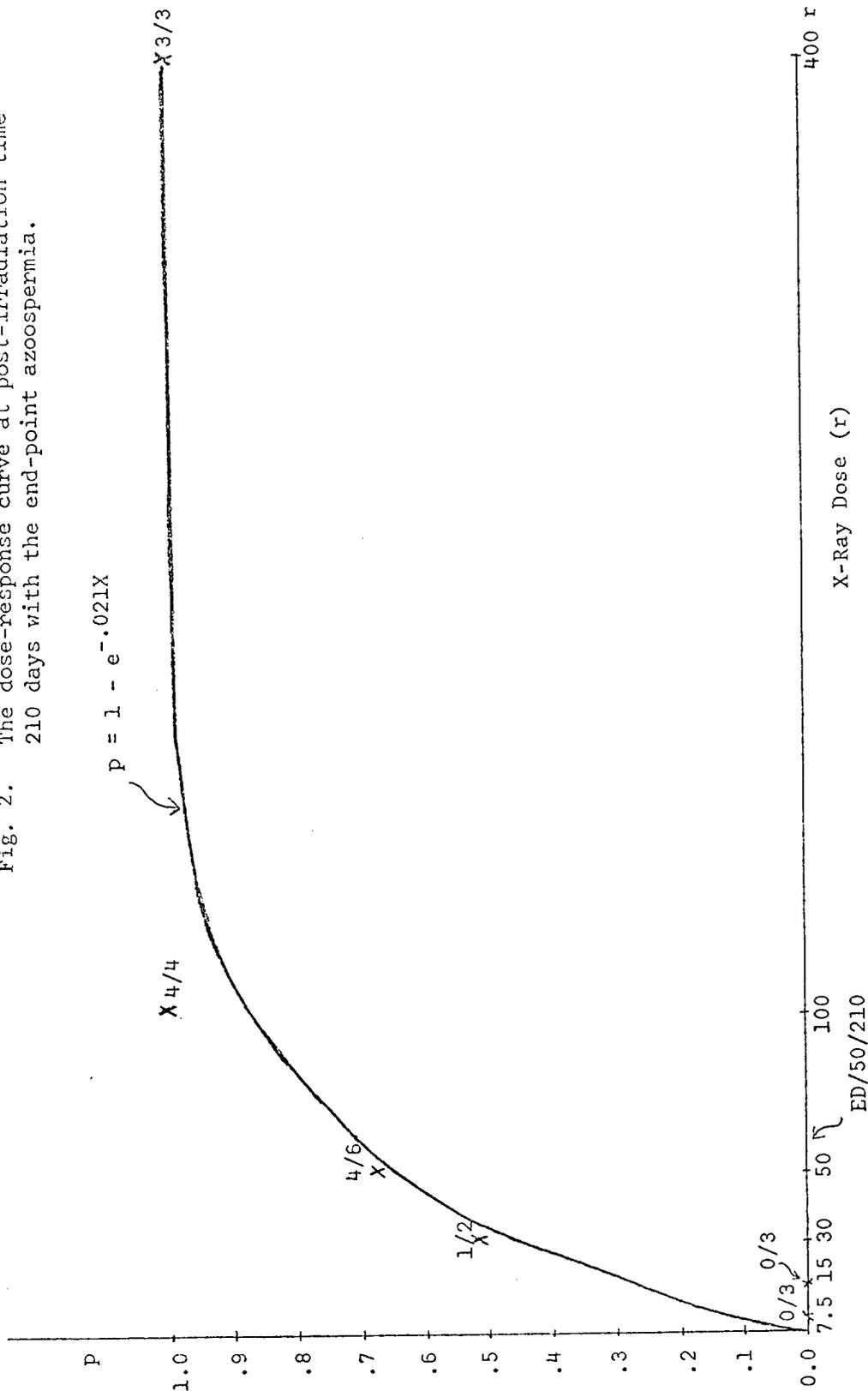
	Time in Days Post-Irradiation				
	90	120	150	180	210
Upper Limit	101.4	64.4	55.3	44.6	21.5
ED/50	61.1	40.1	34.5	28.0	12.7
Lower Limit	20.8	15.8	13.7	11.4	3.9
$\beta$	.01135	.01729	.02007	.02472	.05457

Table 5. ED/50 estimates and .95 confidence interval for the end-point azoospermia at various times post-irradiation.

	Time in Days Post-Irradiation				
	90	120	150	180	210
Upper Limit	1,626.4	276.4	269.7	137.7	55.5
ED/50	682.1	153.3	149.4	80.4	33.0
Lower Limit	0.0	30.3	29.1	23.2	10.4
$\beta$	.00102	.00452	.00464	.00862	.02103

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Fig. 2. The dose-response curve at post-irradiation time 210 days with the end-point azoospermia.



X indicates observed percent affected;  
Na/Ne = number affected/number exposed.

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Table 6. Observed and expected number of affected assuming the model  
 $p = 1 - e^{-.021X}$  for post-irradiation time 210 days with the  
end-point azoospermia

X-ray Dose (r)	Observed Affected	Expected Affected	Number Exposed
400.0	3	2.999	3
100.0	4	3.511	4
50.0	4	3.903	6
30.0	1	0.936	2
15.0	0	0.811	3
7.5	0	0.438	3

$$\chi^2_5 = 2.34$$

$$p \approx .8$$

\* The chi-square value was computed on the basis of both affected and non-affected individuals.

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Until we can create a more appropriate technique for statistical analysis of the germinal epithelium, we are using a method that permits judgement as to major alterations in spermatogenesis. Briefly we count the germinal cells within ten (10) seminiferous tubules cut in cross-section. The tubules selected for quantitation must be circular so that our sampling will not traverse in a tangential fashion through different stages of spermatogenesis. All results are then expressed as the mean specific cell type per tubule.

Due to the differences in life span, as established by Heller and Clermont, some cell types are quite variable in their distribution (e.g. Leptotene spermatocyte) while those with a longer life span are more uniformly distributed throughout the tubules. In the instance of Leptotene spermatocyte, we combined the quantitation of these cells with the Zygotene spermatocytes for better analysis (LZ). Other cells selected for quantitation include the Sertoli cell (S), Adark (Ad) and A pale (Ap) spermatogonia and Pachytene (P) primary spermatocytes.

- B. Material and Methods. Testicular biopsy specimens were obtained before and after irradiation in many but not all of the research subjects. Originally we intended to obtain biopsy specimens on all men prior to irradiation and then at different intervals post-irradiation in order to examine the entire spectrum of damage and renewal. Since we found that the procedure of testicular biopsy caused a reduction in spermatogenesis in some men and that biopsy plus irradiation augmented each other, we changed our procedure to sample men when they had their vasectomy and were leaving the program.

The biopsy specimens were immediately fixed in freshly prepared Cleland's fluid, embedded in paraffin, sectioned serially at 5 micra, and stained with iron-alum hematoxylin and eosin. A low-power photomicrograph (Fig. 3) was made of the entire section. From this photograph all circular tubules were located and numbered. These were then relocated under the microscope and the cell population was counted (Fig. 4) using magnifications of 500 and 1250 X. At least ten such tubules were counted for each biopsy specimen.

For the radiation exposures a 250 KV G.E. Maximar Machine was used to deliver a mid-organ dose of from 7.5 to 400 r (f factor equals 0.95) to both testes. The subject was

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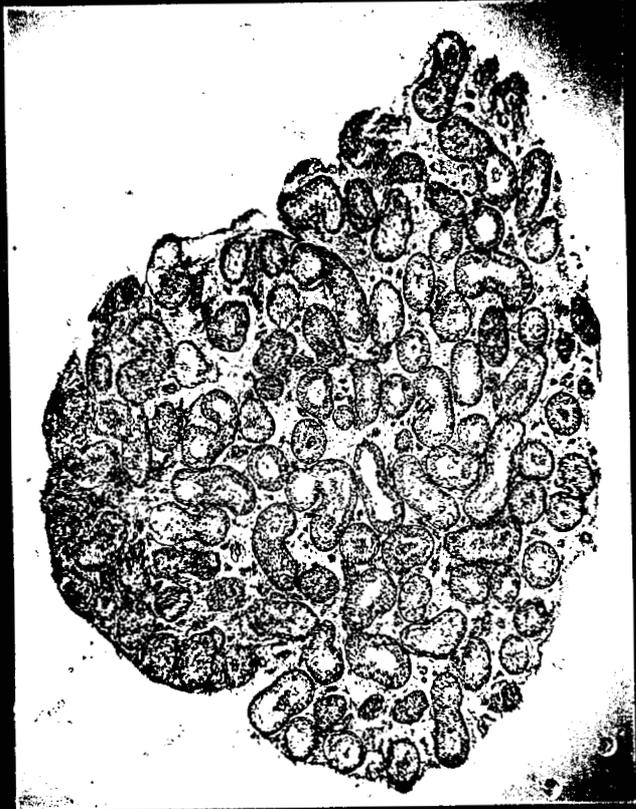


Fig. 3. This is a low-power Polaroid photomicrograph of a biopsy specimen. From this circular tubules are selected and numbered for later identification and scoring under high power magnification. (specimen No. 488-10-3)

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positioned in the supine position with the penis taped upward to the lower abdominal wall. The legs were slightly spread and a plastic-contained bolus of sugar supported the testes. A 10 x 10 cm. cone was used and the FSD was 75 cm. Individual doses included 7.5, 15, 30, 50, 100 and 400 r.

- C. Results. The mean cell count per tubule with the corresponding sperm counts for each of the eighteen normal inmate volunteers obtained from the pre-irradiation period are listed in Table 7. In addition the approximate life span as estimated by Clermont for each cell type are depicted.

Since our aim was to define testicular changes following an acute exposure to irradiation in terms of the general population, the mean counts for the eighteen men served as our frame of reference. In addition, comparisons were made within the same subject when post-irradiation observations were available (Table 8).

Table 9 lists in chronologic order the cell count information following various dose levels of radiation. It can be seen that different doses produced different responses. For example, there was little evidence of damage at 201 to 306 days following 15 r. On the other hand observations from 222 through 264 days post-irradiation demonstrated that 30 r induced a prolonged reduction in the cellular elements of the germinal epithelium. Therefore, the minimal effective dose for producing prolonged damage to spermatogenesis lies somewhere between 15 and 30 r. With respect to recovery, our observations at 887 days following 30 r indicate that testicular histology had returned to normal. The same was true for 50 r at 609 and 973 days post-irradiation.

At 50 r consistent azoospermia did not occur. However, with 100 r, uniform changes in testicular histology, sperm counts and urinary gonadotrophin excretion levels were noted. Therefore, this dose was selected to make a more detailed examination of changes in testicular histology and physiology following x-ray irradiation.

After exposure to 100 r serial observations were obtained in a group of volunteers at varying time intervals up to 1219 days. These time intervals were grouped for analysis into periods which revealed a similar pattern of histologic damage and recovery. The results are as follows:

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Table 7 NORMAL GERM CELL POPULATION, AVERAGE COUNTS PER TUBULE

RV	Sertoli	A dark	A pale (16)*	Leptotene- Zygotene (7)*	Pachytene (16)*	Sperm Count Million/cc	
						Mean	Range
2	10.2	13.2	4.9	9.2	21.2	74	18-147
7	10.6	13.2	3.7	11.2	27.9	133	55-198
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	82-285
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
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
Mean	14.9	15.1	6.9	12.1	33.0		
S.D.	3.7	4.1	3.8	3.1	6.9		
CV	24.8	27.2	55.1	25.6	20.9		

\* Digits within the parenthesis refer to life span in days.

1001926

Table 8 INDIVIDUAL CONTROL BIOPSY CELL COUNTS COMPARED WITH POST-IRRADIATION

RV	Days After Irradiation	Sertoli	A Dark	A Pale	Leptotene- Zygotene	Pachytene
15 r						
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
	305 days	18.9	15.5	5.8	9.3	18.0
50 r						
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 "	16.4	26.2	9.5	17.0	33.3
100 r						
38	Control	18.1	18.2	6.2	19.0	37.3
	9 days	16.2	11.3	3.3	9.5	18.6
48	Control	12.5	15.2	5.5	12.6	19.6
	15 days	17.3	8.2	3.5	1.1	16.9
42	Control	15.4	12.7	5.8	13.8	32.7
	15 days	16.0	8.6	4.0	3.3	9.1
16	Control	9.1	16.0	4.8	9.1	35.5
	15 days	14.1	14.2	4.5	5.3	22.5
32	Control	13.0	7.8	17.7	14.0	37.0
	33 days	19.2	9.2	4.8	5.1	10.1
43	Control	17.2	19.2	5.7	12.8	32.1
	33 days	21.8	12.5	6.5	1.9	8.9
47	Control	16.2	20.3	4.6	11.9	41.2
	33 days	22.2	10.2	3.5	0.2	3.9
53	Control	13.9	11.8	5.9	10.3	37.7
	49 days	18.2	5.8	3.2	2.2	6.2
7	Control	10.6	13.2	3.7	11.2	27.9
	49 days	23.6	7.5	3.2	0.5	2.9
2	Control	10.2	13.2	4.9	9.2	21.2
	49 days	21.7	4.0	2.5	0.7	1.3
400 r						
14	Control	16.4	17.8	3.5	9.2	47.2
	91 days	25.9	0.3	0	0	0
	1540 "	17.8	15.9	2.5	13.5	33.3
63	Control	14.9	11.2	3.0	10.5	26.1
	Immediately	15.7	10.9	3.8	6.8	12.6

1001927

Table 9 POST-IRRADIATION CELL COUNTS RELATED TO DOSE AND TIME INTERVAL

RV	Days After Irradiation	Sertoli	A dark	A Pale	Leptotene-Zygotene	Pachytene
Mean of 18 Normal Controls		14.9	15.1	6.9	12.1	33.0
<b>15 r</b>						
154	175 days	15.6	7.8	0.2	1.2	5.0
39	201 "	16.3	10.3	1.8	4.5	21.0
41	305 "	25.6	11.9	5.3	8.1	16.9
18	306 "	18.9	15.5	5.8	9.3	18.0
<b>30 r</b>						
21	159 "	25.3	2.6	2.0	2.4	1.3
30	222 "	17.9	3.5	1.5	2.7	4.6
34	264 "	19.0	6.7	2.9	3.1	7.2
29	887 "	14.9	12.8	2.8	11.5	24.0
<b>50 r</b>						
17	609 "	19.6	17.8	14.8	13.2	26.4
17	973 "	16.4	26.2	9.5	17.0	33.3
<b>100 r</b>						
79	Immediately	17.0	11.8	2.9	14.8	35.1
38	9 days	16.2	11.3	3.3	9.5	18.6
54	14 "	23.1	13.3	5.2	5.8	23.5
48	15 "	17.3	8.2	3.5	1.1	16.9
42	15 "	16.0	8.6	4.0	3.3	9.1
16	15 "	14.1	14.2	4.5	5.3	22.5
28	24 "	18.0	8.7	5.1	4.5	11.9
27	27 "	13.7	7.7	5.3	1.0	5.2
32	33 "	19.2	9.2	4.8	5.1	10.1
43	33 "	21.8	12.5	6.5	1.9	8.9
47	33 "	22.2	10.2	3.5	0.2	3.9
53	49 "	18.2	5.8	3.2	2.2	6.2
7	49 "	23.6	7.5	3.2	0.5	2.9
2	49 "	21.7	4.0	2.5	0.7	1.3
2	153 "	18.4	1.0	0.4	0	0
47	228 "	18.9	1.5	0.8	0	0
42	280 "	16.9	5.5	1.9	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
3	973 "	11.3	14.9	4.8	16.9	32.6
38	1017 "	16.7	16.7	1.8	11.7	41.9
6	1022 "	13.2	16.4	5.6	15.7	29.6
27	1050 "	8.8	12.5	3.8	10.0	16.3
16	1219 "	14.3	22.3	3.2	7.5	31.6
<b>400 r</b>						
23	Immediately	11.9	13.0	6.1	12.3	27.9
63	"	15.7	10.9	3.8	6.8	12.6
63	63 days	18.4	4.2	0.6	0	0
14	91 "	25.9	0.3	0	0	0
22	188 "	23.3	1.1	1.1	0.6	0.8
22	355 "	20.2	6.6	2.1	3.4	7.9
5	450 "	15.0	2.7	1.2	2.4	7.9
26	503 "	16.1	3.3	1.5	5.5	6.4
23	967 "	22.4	0.5	0.2	0.2	1.3
14	1540 "	17.8	15.9	2.5	13.5	33.3

1001928

- 1) At 14-15 days there was a clear cut reduction in the Leptotene - Zygotene primary spermatocytes. Existing information suggests that the B spermatogonia is the most sensitive cell to radiation effects. Since the type B spermatogonium is the immediate precursor to the L-Z cells, the reduction in L-Z spermatocytes observed at this time interval indicates that the type B spermatogonia were directly damaged by exposure to 100 r.
- 2) At 33 days (Fig. 5) the previously noted reduction in the L-Z spermatocytes is now reflected by a significant reduction in the numbers of Pachytene primary spermatocytes. Instead of indicating direct radiation damage to the primary spermatocytes these data demonstrate the damage occurred to a cell population which was "33 days younger". At this time interval it would be the type B spermatogonia and possibly the A pale spermatogonia.
- 3) At 49 days (Fig. 6) the progressive depletion of the L-Z and P Primary spermatocytes reflects two phenomena: (a) the initial damage to B spermatogonia and (b) the inability of the A pale spermatogonia to replenish the "B" cell population. If only the B spermatogonia were initially damaged by irradiation one would expect the B spermatogonia to be replenished by a new generation of Ap cells. Along this same theme, the L-Z cell population should be recovering by now if the Ap cells had not been damaged along with the type B spermatogonia.
- 4) Observations made at 153 and 228 days (Fig. 7, 153 days) demonstrate the almost complete absence of germinal cells, with only Sertoli cells remaining. No primary spermatocytes could be detected and only an occasional spermatogonium could be identified. This marked reduction in numbers of both Ap and Ad spermatogonia clearly indicates that the A dark spermatogonia were also directly damaged at the time of irradiation. It may be that at this time interval those cells being "scored" as Ad are, in fact, the so-called radio-resistant "stem-cells" or Ao which have been considered to be present in rats, but so far not specifically identified in the human.

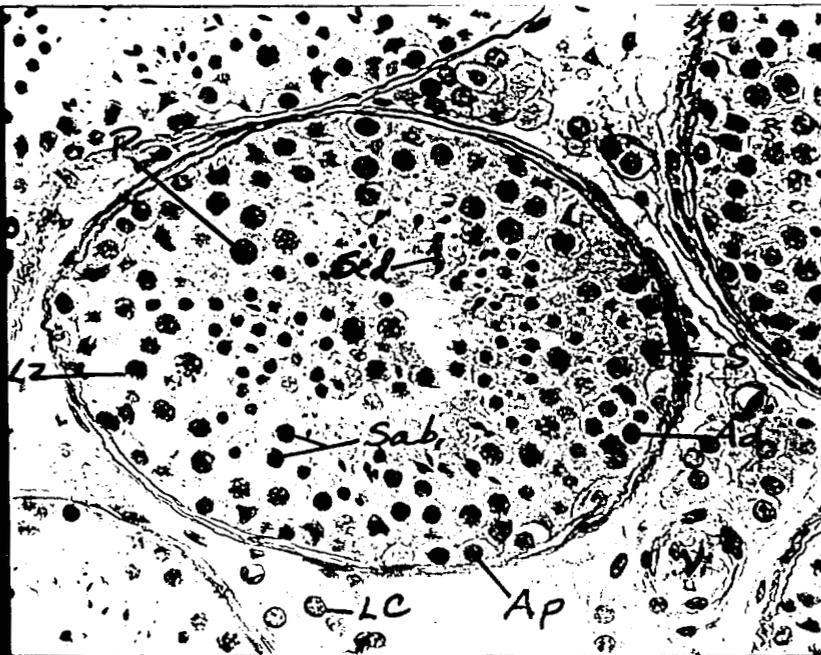


Fig. 4. Normal Control Biops

- S = Sertoli cell
- Ad = A dark spermatogonium
- Ap = A pale spermatogonium
- LZ = Leptotene-Zygotene primary spermatocyte
- P = Pachytene primary spermatocyte
- LC = Leydig cell
- V = Blood vessel
- Sabl = Early spermatids
- Scd = Maturing spermatids

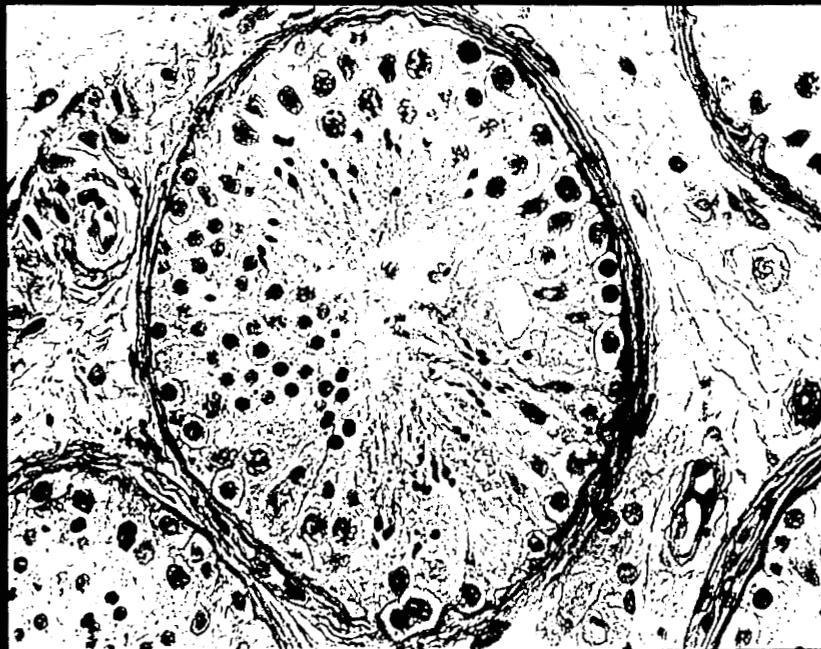


Fig. 5. 33 days after 100 X-ray to the testi

No significant change in Sertoli cell population.  
Decrease in Leptotene-Zygotene and Pachytene primary spermatocytes

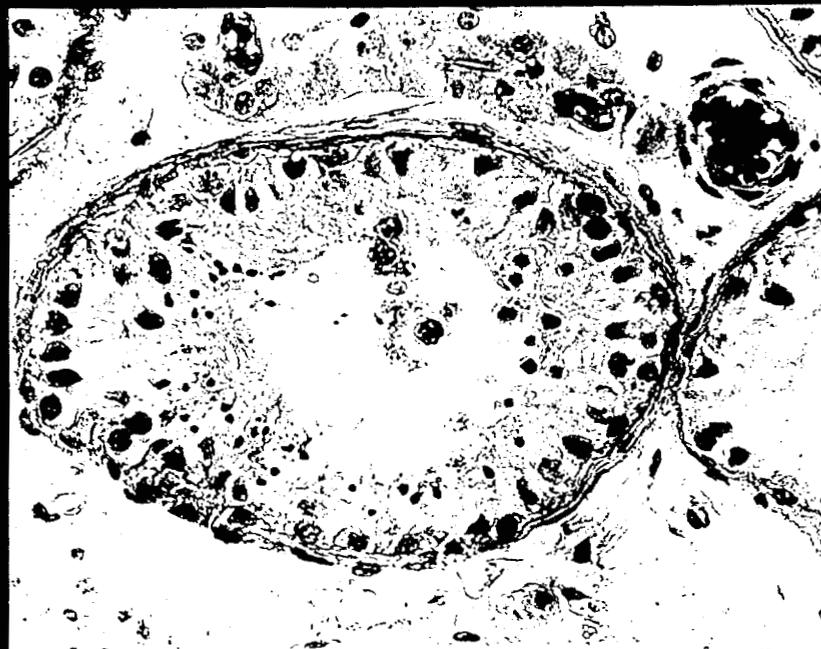


Fig. 6. 49 days after 100 r X-ray to the testis

Marked decrease in LZ and P primary spermatocytes.  
Early decrease in Ad spermatogonia

1001930



Fig. 7.

153 days after 100 r to the Testis

Virtual loss of all germinal elements

Leydig cells normal in appearance



Fig. 9.

466 days after 100 r

Definite evidence of histologic recovery. Most germ cell elements present in varying numbers.

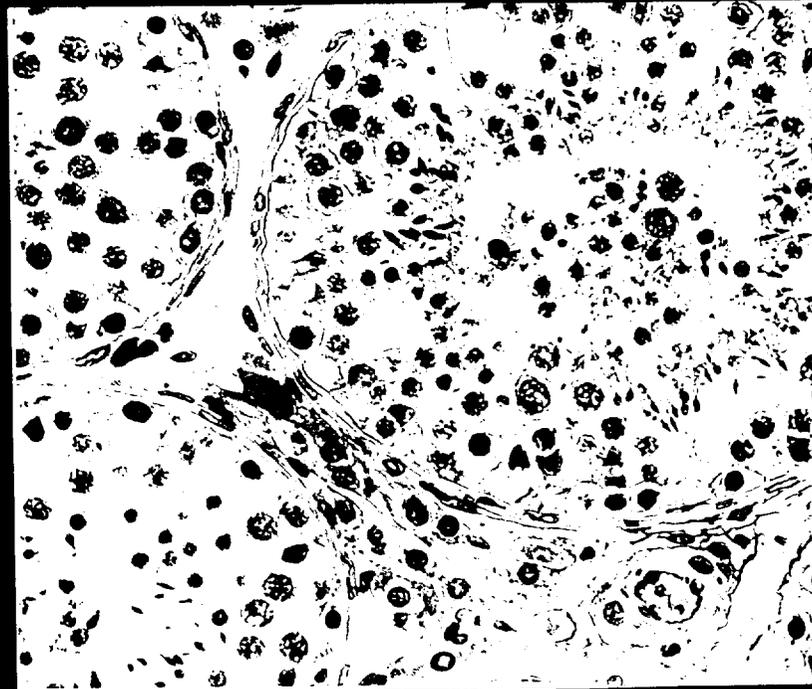


Fig. 10.

1022 days after 100 r

Full, normal spermatogenesis present in most tubules. No striking evidence of peritubular or vascular hyalinization. Sperm count has returned to control range.

- 5) The intervals of 280 and 311 days post-irradiation reveal evidence of germinal epithelial repopulation. In particular it is interesting to note that there is a four-fold increase in numbers of Ad spermatogonia (Fig. 8) maturation has not yet proceeded beyond this point to any significant extent. This would indicate that the stem-cell line has resumed mitotic activity. Furthermore, the data results indicate that a minimum of two successive divisions have occurred within a 52 to 83 day period. This suggests that the life span for the Ad or stem-cell spermatogonia does not exceed 26 days.

Finally, the logarithmic growth for the Ad spermatogonia continues through the 466th post-irradiation day (Fig. 9). At this time, control levels of Ad spermatogonia are reached and then the more mature cells rapidly increase in numbers.

- 6) Between 737 and 1219 days there were multiple observations made. Quantitative results indicate that complete recovery of the germinal epithelium had occurred and this was maintained (Fig. 10).

Two biopsy specimens were obtained immediately after 400 r. These specimens did not show any alteration in cell counts. Therefore, if immediate cell killing occurred as a consequence of 400 r it was not clearly evident. By 63 and 91 days fairly complete denudation of the germinal elements were observed (Figs. 11-16). Multiple observations were made between 188 and 967 days which showed that the damage was sustained (Table 9). However, by 1540 days complete histologic recovery was documented (Fig. 16).

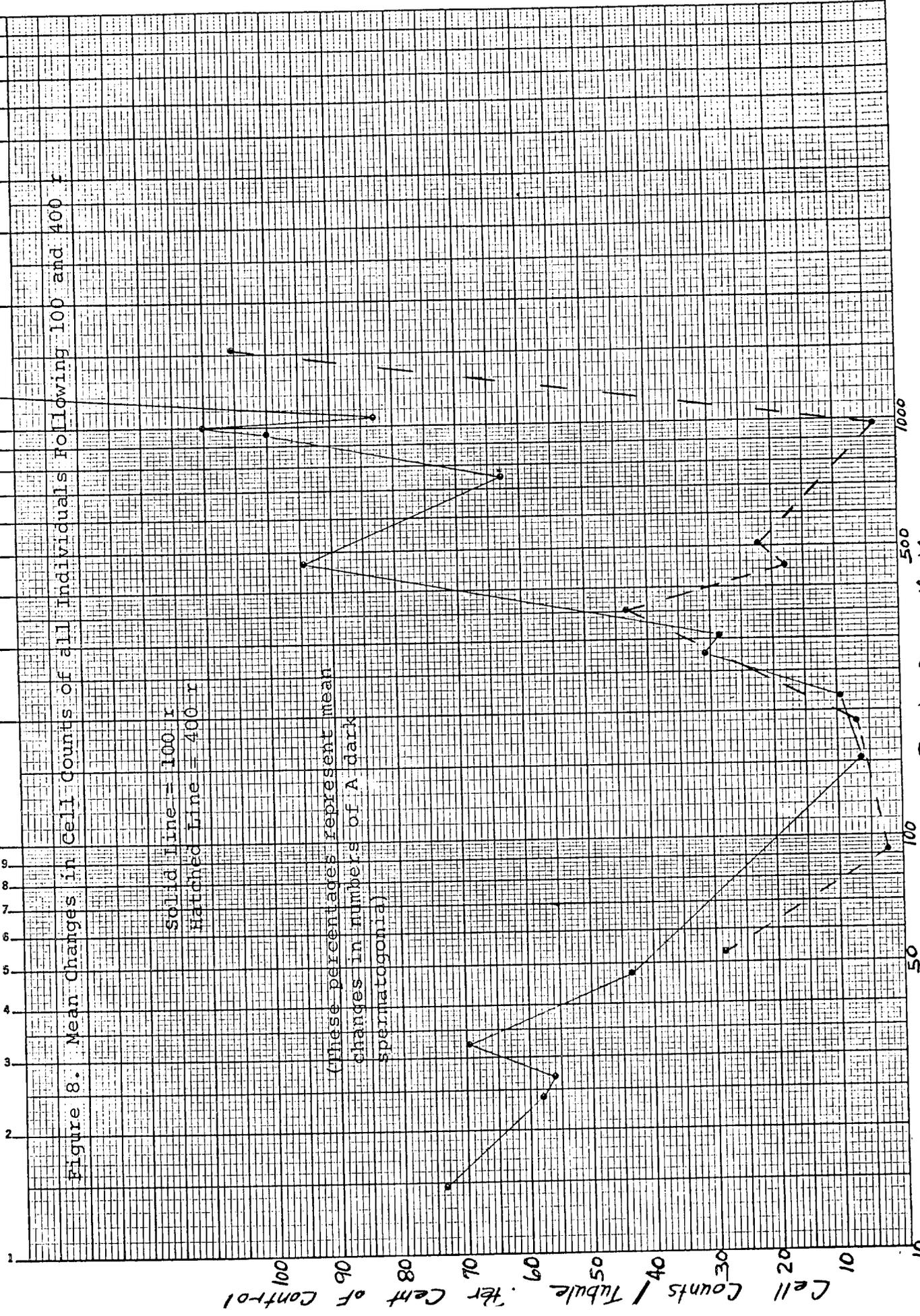
The observations with respect to the Ad spermatogonia at the 100 r dose level were similar when we examined the 400 r data. This emphasizes the suggestion that the Ad spermatogonia preferentially restores its own population before further cellular maturation can be observed (i.e. an significant increase in Ap spermatogonia). These data also permit us to estimate that the life span of the Ad spermatogonium is not more than 26 days. In view of Clermont's work on the kinetics of spermatogenesis it is likely that the Ad cell adheres closely to the 16-day cycle proposed for the germinal epithelium.

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Figure 8. Mean Changes in Cell Counts of all Individuals Following 100 and 400 r

Solid Line = 100 r  
Hatched Line = 400 r

(These percentages represent mean changes in numbers of A dark spermatogonia)



Days Post Irradiation

1001933

Fig. 11.

RV-23 Control biopsy

See fig. 4 for comparison

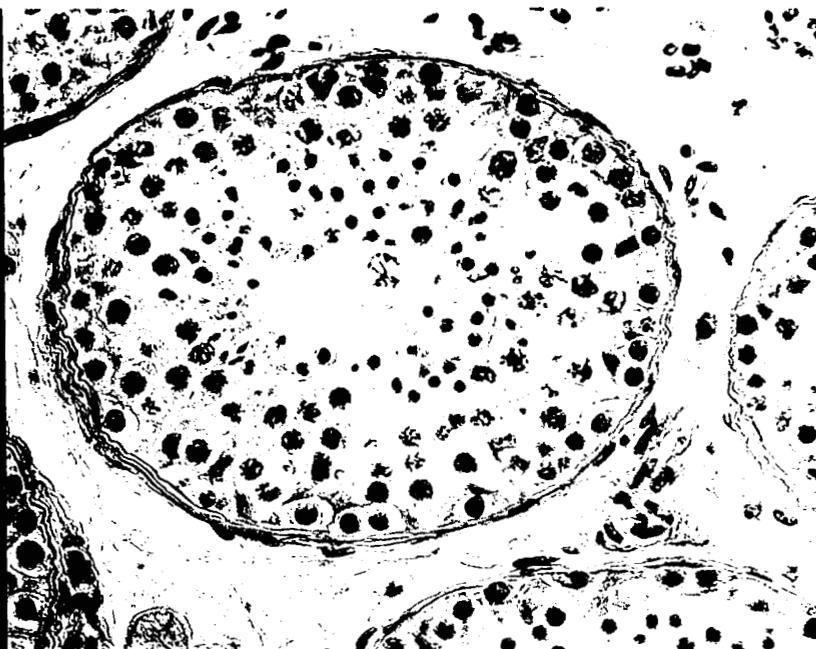


Fig. 12

RV-23 91 days after 400 r

Virtual absence of germinal epithelium. Only occasional spermatogonia seen. Sertoli cells prominent. Absence of primary spermatocytes. Compare with fig. 7, at 153 days following 100 r.

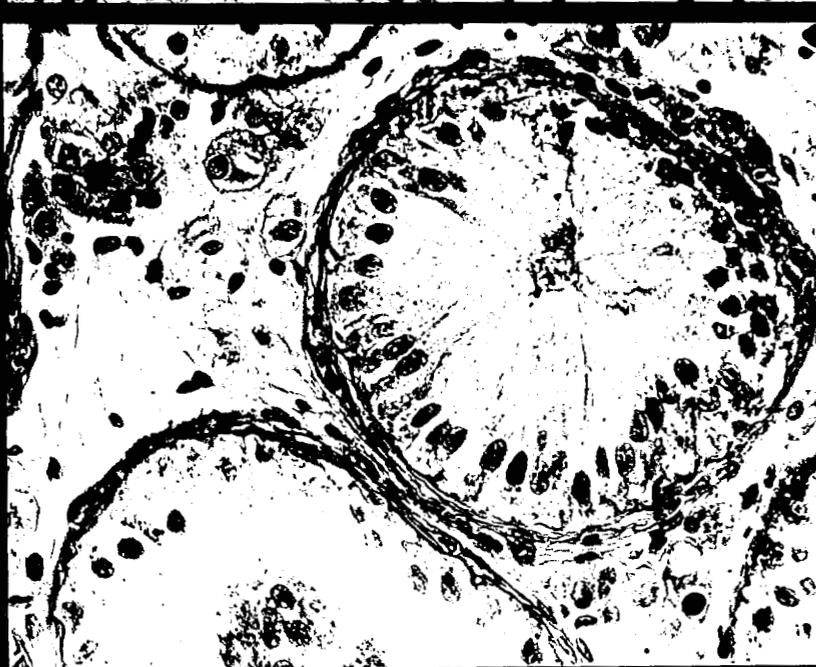
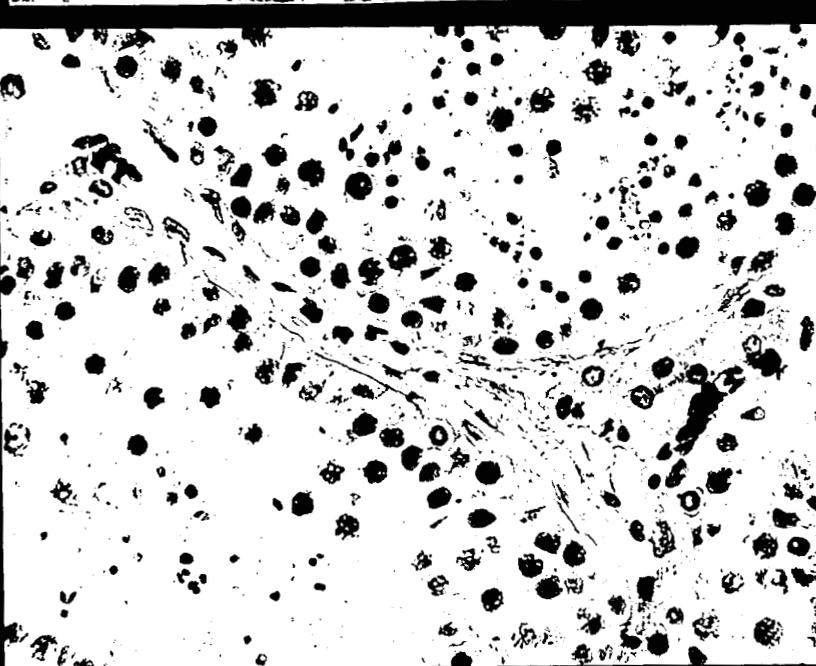


Fig. 13

RV-23 967 days after 400 r

Beginning recovery of germinal epithelium in some tubules, but not in others. Extent of recovery at this interval is comparable to that seen 466 days after 100 r (fig. 9).



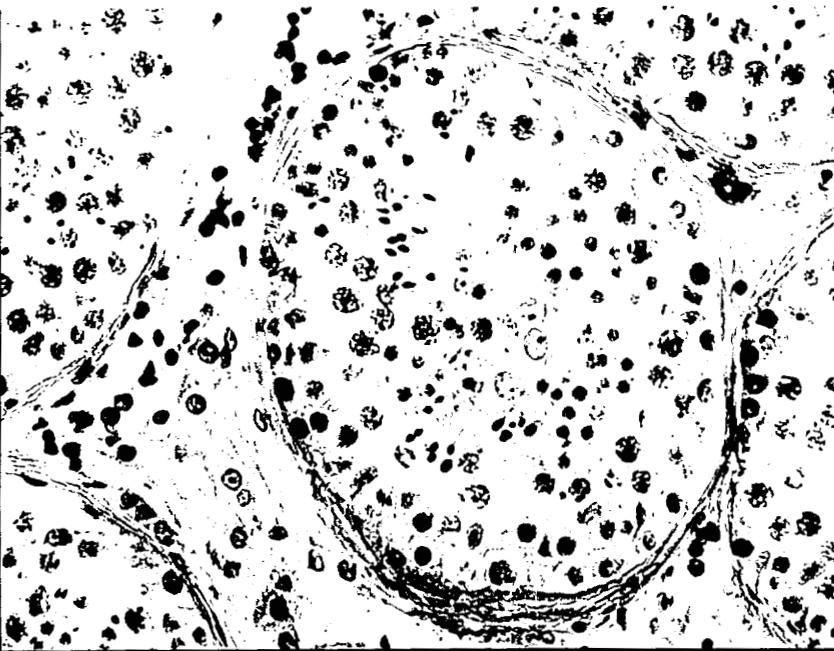


Fig. 14

RV-14 Control biopsy

Compare with figures 4 and 11

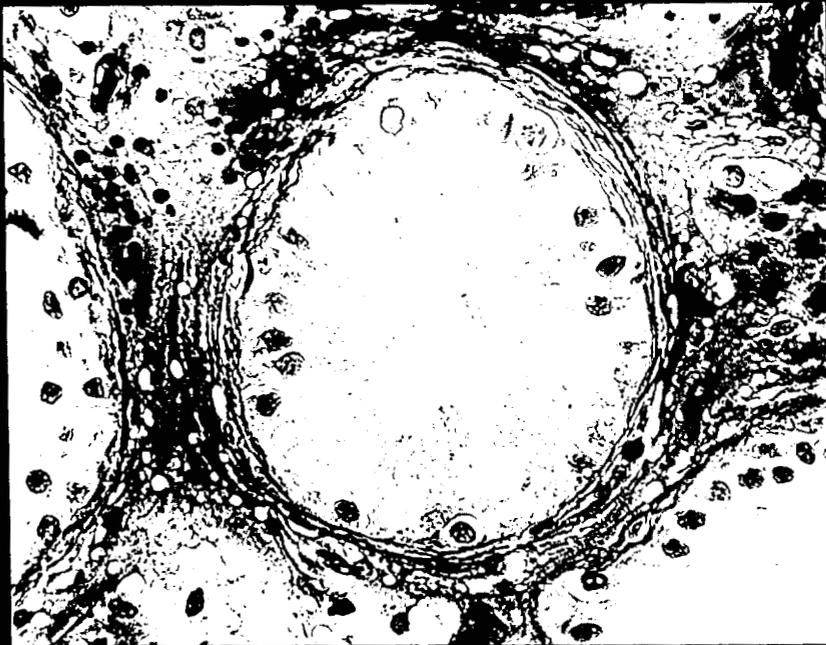


Fig. 15

RV-14 91 days after 400 r

Compare with fig. 12 at same dose and time interval, and with fig. 7, 153 days after 100 r.

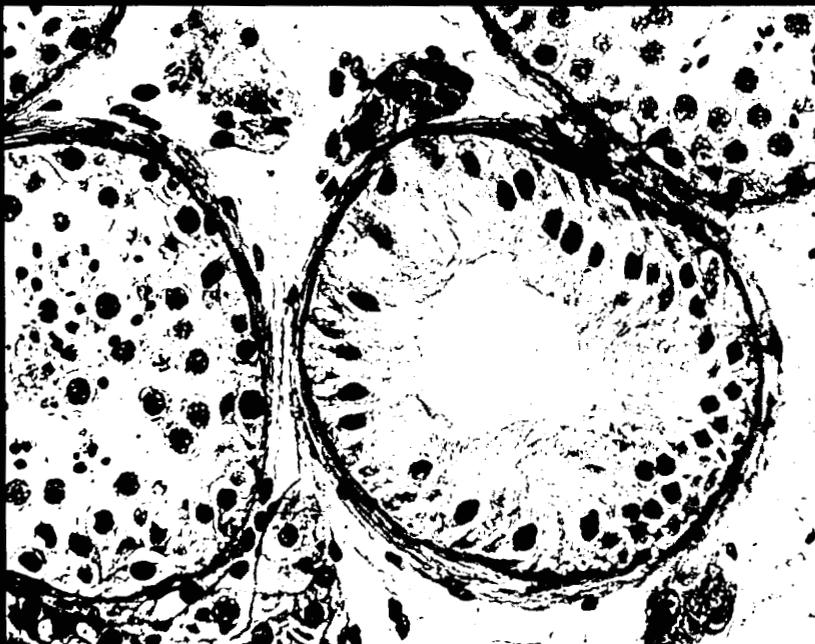


Fig. 16.

RV-14 1540 days after 400 r

Full recovery of spermatogenesis in most tubules. Comparable to recovery from 100 r at 1022 days (Fig. 10).

To summarize our observations the data are plotted graphically for all cell types (Fig. 17). The changes in Sertoli cell counts probably reflect changes in tubular wall length and diameter following irradiation.

Finally, minimal vascular changes were noted 91 days after 400 r (Fig. 18,19). We have not completed our evaluation of the vascular system but it appears that doses of 100 r or less do not produce significant fibrinoid changes.

100193b

FIGURE 17. CHANGES IN CELL COUNTS PER TUBULE FOLLOWING 100 R.

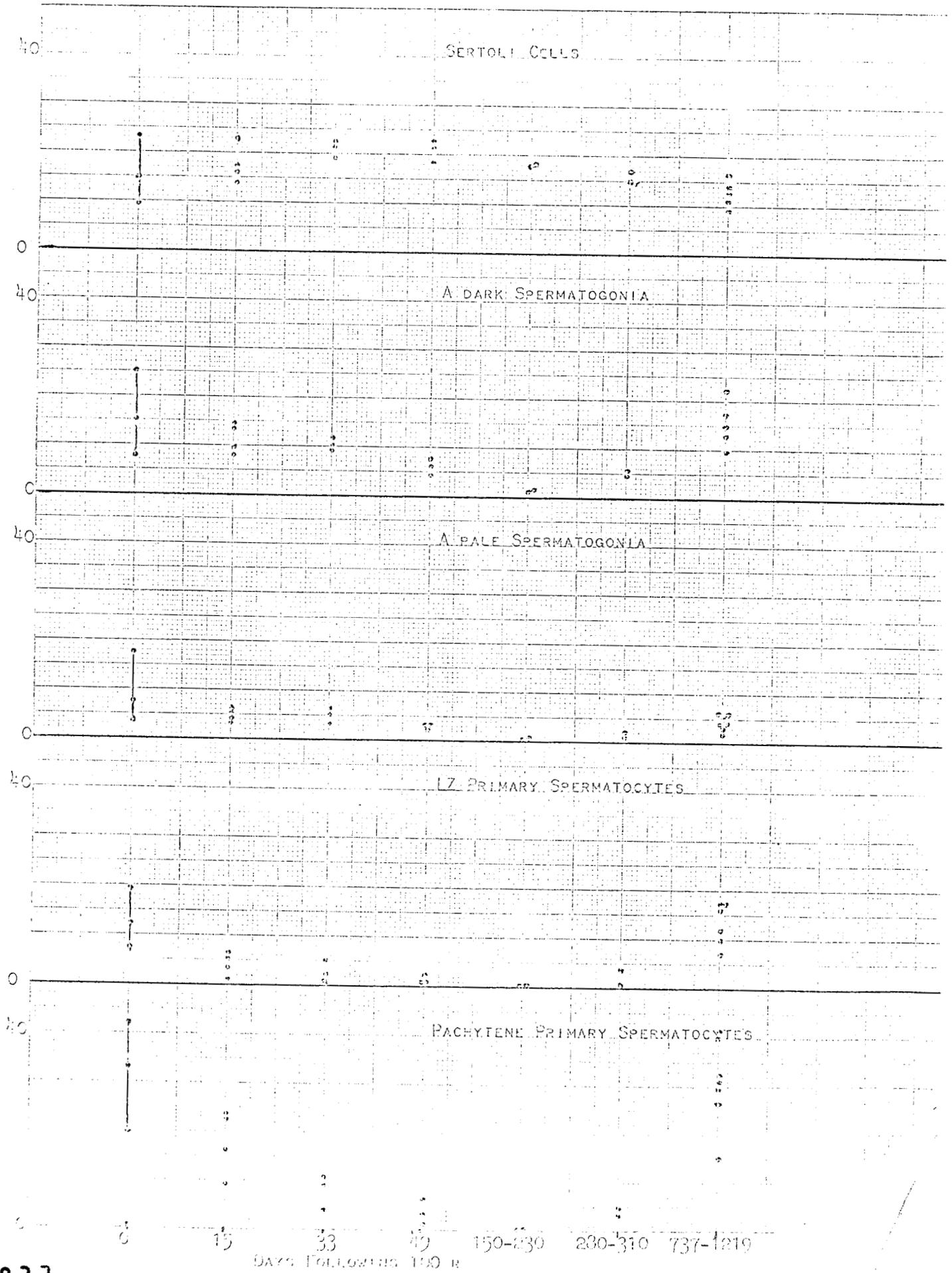
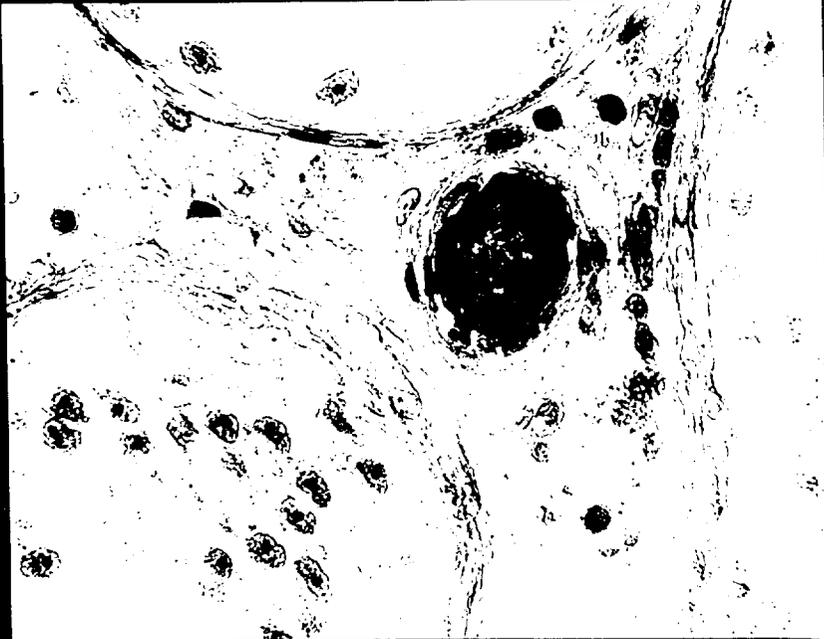


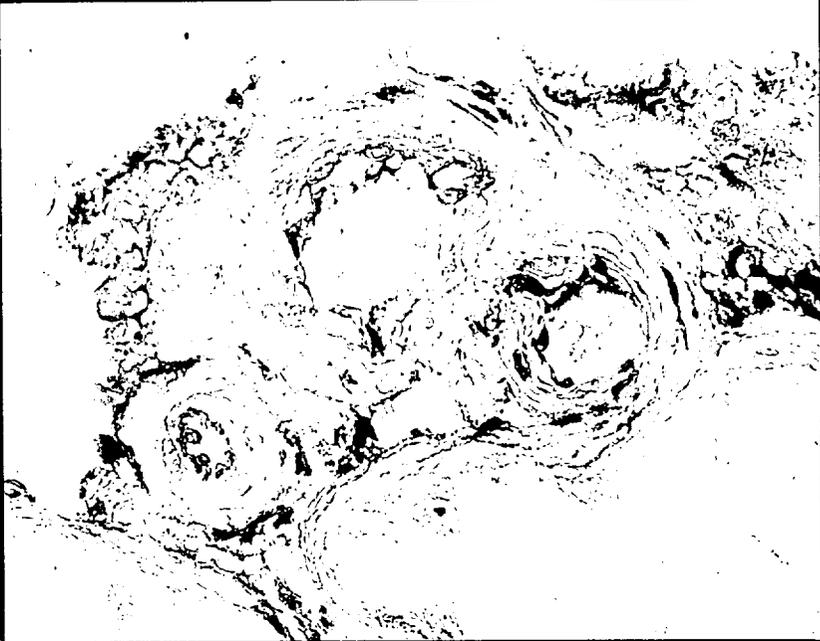
FIG. 17. 10 X 10 TO THE CENTIMETER 47 1510  
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Figs. 18 and 19.

Blood vessels 91 days after 400 r

Fig. 18 shows a normal appearing vessel, while fig. 19 shows some evidence of variable fibrinoid changes, such as those noted by Scott et al. in the Hiroshima and Nagasaki study, ABCC technical report 15-66



IV. Chromosomal Studies

A. Mitotic Chromosomes

1. Material and Methods

Numerous reports have documented the fact that chromosomal damage occurs following in vitro irradiation. Similar changes have been noted following in vivo irradiation. The purpose of our study was to explore the dose-response relationship of such damage following x-ray irradiation to the testes in vivo. Testicular tissue obtained by biopsy and grown in short-term monolayer culture was analyzed for chromosomal changes. Specimens were obtained prior to and at varying intervals following irradiation. These post-irradiation time intervals included four hours, fifteen days and two and three-quarter years. Since it was not possible to obtain pre-irradiated testicular biopsy specimens in each subject, our control data were pooled. Except for two instances, 100 r was the dose of x-ray utilized. The exceptions included one subject exposed to 30 r and one exposed to 400 r. In addition, chromosomal analyses were performed on peripheral blood lymphocytes obtained from specimens collected prior to irradiation and at 30 minutes, 4 hours and 15 days following x-ray irradiation.

The blood was cultured by a modification of the method of Moorehead. Phytohemagglutinin-P was added to the heparinized blood. This mixture was allowed to stand in an ice bath for twenty-five minutes after which it was centrifuged at 25 g's. for five minutes. Two to three ml of the cell-rich plasma were added to a two ounce medicine bottle containing TC199, penicillin (100 units/ml), streptomycin (100 µg/ml) and mycostatin (10 units/ml); then the material was incubated at 37 degrees under 5% CO<sub>2</sub> tension for 72 hours. At the time the cells were "harvested" colcemid (0.2 µg/ml) was added to the culture for four hours. Following this procedure the cells were placed into a hypotonic solution for 15 minutes and then "fixed" in acetic acid - methanol (1:3) for thirty minutes. After fixation the cells were suspended into the fixative solution and dripped onto cold wet slides, allowed to air dry and then stained with tetrachrome.

1001939

Testicular tissue was cultured by a modification of the method of Steinberger and Steinberger. The testicular cells which are analyzed by this technique are thought to be interstitial rather than germinal in nature. This consideration is based on the studies of the Steinbergers which demonstrated the presence of 3- $\beta$ -al dehydrogenase in testicular cells grown in monolayer cultures. In preparation for culture, the testicular tissue was minced in 0.25% trypsin and then placed into a 37 degree incubator for 15 minutes. The trypsinized tissue was then placed into the nutrient media at a cellular suspension concentration of one million cells per ml. The nutrient media contained TC 199, 20% fetal calf serum and antibiotics (penicillin, 100 units/ml and streptomycin, 100  $\mu$ g/ml). The cellular suspension was plated onto cover slips within Leighton tubes and incubated at 37 degrees under a 5% CO<sub>2</sub> tension for a period of 7-10 days. The cultures were then harvested in a similar fashion to the peripheral blood cultures.

Each slide was scanned for the purpose of locating 50 suitable metaphase plates. These cells were then photographed, karyotyped and analyzed. Such preparations were scored separately by three investigators for abnormalities which included breaks, acentric fragments, dicentrics, rings, translocations, deletions and inversions.

## 2. Results

a) Introduction. From our two control blood specimens 44 metaphase plates were karyotyped. From our six post-irradiated specimens 82 metaphase plates were karyotyped. The data derived from these preparations revealed that there was no increase in chromatid or chromosomal abnormalities induced in the peripheral blood of subjects whose testes were irradiated with x-ray. This was anticipated and serves to rule out technical difficulties in our study.

Twenty-one control testicular cultures produced 770 cells suitable for analysis. From seven post-irradiation biopsy specimens the tissue culture preparations produced 311 cells suitable for analysis and comparison. These data are listed in Tables 10 and 11.

1001940

b. Results and Statistical Analysis of Chromosome Breakage

The first step in the development of a test criterion to detect an increase in chromosome breakage is to postulate a probability distribution for the number of breaks in a control cell.

If we assume that there are a very large number of locations  $N$  where a chromosome break may occur and that the probability of a break may occur at each location is  $p$ , then if the breaks are acting independently of each other, the probability distribution of the total number of breaks  $x$  is the poisson.

$$(1) \quad p\{x = v\} = \frac{e^{-\lambda} \lambda^v}{v!} \quad v = 0, 1, \dots$$

where  $\lambda = Np$ .

If we look at  $\xi_1$  cells and count the total number of breaks  $x_1$  in the  $\xi_1$  cells, then  $x_1$  also has a poisson distribution but with parameter  $\lambda\xi_1$ . Thus,

$$(2) \quad p\{x_1 = v_1\} = \frac{e^{-\lambda\xi_1} (\lambda\xi_1)^{v_1}}{v_1!} \quad v_1 = 0, 1, \dots$$

If we also count the number of chromosome breaks  $x_2$  in  $\xi_2$  cells of a person who has been irradiated and if there has been no radiation effect, then the probability distribution of  $x_2$  is

$$(3) \quad p\{x_2 = v_2\} = \frac{e^{-\lambda\xi_2} (\lambda\xi_2)^{v_2}}{v_2!} \quad v_2 = 0, 1, \dots$$

Let us assume that we have counted  $v_1$  breaks in  $\xi_1$  cells and  $v_2$  breaks in  $\xi_2$  cells and we wish to know, assuming no radiation effect, the probability of this occurring by chance alone. It can

1001941

be shown that this is equal to

$$(4) \quad p\{x_2 \geq v_2/v_2 + v_1\} = x_2^{\frac{v_2+v_1}{x_2}} \binom{v_2+v_1}{x_2} \left(\frac{\xi_2}{\xi_2+\xi_1}\right)^{x_2} \left(\frac{\xi_1}{\xi_2+\xi_1}\right)^{v_2+v_1-x_2}$$

If  $v_1 = 0$  then equation (4) reduces to

$$(5) \quad p\{x_2 \geq v_2\} = \left(\frac{\xi_2}{\xi_2+\xi_1}\right)^{v_2} .$$

Equation (4) may be evaluated by using the "F" tables and the fact that

$$(6) \quad \frac{v_2}{v_1+1} \frac{\xi_1}{\xi_2} \sim F[2(v_1+1), 2v_2] .$$

Since individuals vary in regard to the extent of their natural chromosome breakage it is highly desirable to allow each individual to act as his own control. This type of data was obtained for four individuals, and the results are shown in Table 10. The interpretation of the "p" value is that under the assumption that there has been no radiation effect an event has occurred that has a probability p. If p is less than an agreed-upon value  $\alpha$  then we reject the hypothesis of no radiation effect. The value  $\alpha$  is the Type I error and by convention is usually taken as .05 or .01.

For several cases a limited number of karyotypes were obtained. Thus, our ability to detect a change in these cases, when in fact it did occur, is relatively small. In other words, for these cases the power of the statistical test is small or the Type II error is large.

A less desirable type of control to use is the pooled data from a number of individuals. However, in the absence of another alternative this approach does have considerable utility. It should be kept in mind, since each individual is compared to the same control, that the results obtained for each individual are not independent of each other.

A total of 770 karyotypes from 21 subjects were examined and a total of 28 breaks were found. These data were used as a control and compared to each of the post-irradiation biopsy material. This comparison is shown in Table 11. We note that the significance levels are much lower than those obtained in Table 10.

In summary our data support the concept that irradiation to tissue produced chromosomal abnormalities. However, the validity of the probability statements depend on the homogeneity of chromosomal breakage in the normal population. This point has not been fully defined.

1001943

TABLE 10  
DETECTION OF INCREASE OF CHROMOSOME BREAKAGE USING INTERNAL CONTROL

RV#	CONTROL		POST-IRRADIATION				$p = \left( \frac{\xi_2}{\xi_2 + \xi_1} \right)^{v_2}$	PROBABILITY OF HAVING $v_2$ BREAKS BY CHANCE ALONE (assuming no radiation effect)
	#BREAKS	#KARYOTYPES (CELLS)	X-RAY DOSE	TIME POST- IRRADIATION	#BREAKS	#KARYOTYPES (CELLS)		
	$v_1$	$\xi_1$				$\xi_2$		
29	0	3	30R	2 yrs. 5 mo.	11	56	.578	
16	0	6	100R	4 hrs. 2 weeks	3 6	41 27	.664 .301	
6	0	20	100R	4 hrs.	6	42	.097	
3	0	14	100R	10 mo.	14	54	.040	

1001944

TABLE 11

## DETECTION OF INCREASE OF CHROMOSOME BREAKAGE USING EXTERNAL CONTROLS

RV#	X-RAY DOSE in rads	TIME POST-IRRADIATION of biopsy	#BREAKS $v_2$	# KARYOTYPES (CELLS) $\xi_2$	$F^* = \frac{v_2 \xi_1}{(v_1 + 1) \xi_2}$	P
29	30R	2 yrs. 5 mo.	11	56	5.214	<.005
16	100R	4 hrs. 2 weeks	3 6	41 27	1.941 5.899	N.S. <.005
6	100R	4 hrs.	6	42	3.791	<.01
3	100R	10 mo.	14	54	6.884	<.005
32	100R	3 yrs.	19	58	8.695	<.005
38	100R	2 yrs. 9 mo.	9	52	4.593	<.005
23	400R	2 yrs. 8 mo.	0	23	0	N.S.

N.S. Non-significant implies  $p > .1$ Control  $\xi_1 = 770$ ,  $v_1 = 28$ 

1001945

B. Meiotic Chromosomes

1. Normal Non-Irradiated Adult Males. The following constitutes a manuscript under preparation for submission to the American Journal of Human Genetics.

STRUCTURAL ABNORMALITIES OF GERM CELL

CHROMOSOMES IN MEN WITH NORMAL SPERMATOGENESIS

by

Stephen P. Swersie and C. Alvin Paulsen

INTRODUCTION

Spermatogenesis in the adult male passes through several stages of development before production of mature spermatozoa. Theoretically, four spermatids are produced from each initial spermatocyte by the process of meiosis. Recent data, however, indicate that an attrition rate of 37% may actually be present during human meiosis (Barr, 1967). Study of the meiotic process in the human male has been infrequent. Evaluation of germ cell chromosomes in man was first attempted by von Winiwarter (1912), using histologic sections. Painter (1923), using a similar technique, later stated that 48 chromosomes were normally present at spermatogonial metaphase. It was only after a consistent chromosome count of 46 was reported in embryonic lung tissue (Tjio & Levan, 1956) that the true chromosome number in man became known. Technical improvements in methodology have subsequently permitted better quantitative evaluation of the germ cells of man. Additional information regarding the morphologic variations of human meiotic chromosomes has accumulated since 1956. These reports, unfortunately, have all been obtained from men with either genitourinary pathology or from subjects in which the status of spermatogenesis was undefined. (Ferguson-Smith 1961; Darlington & Haque, 1962; Book & Kjessler, 1964; Sasaki & Makino 1965; McIlree et al., 1966; Hulten et al., 1966; Kjessler, 1966; McDermott, 1966).

The present report describes the germ cell chromosomes in adult males with verified normal spermatogenesis. Chromosomes were examined

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for structural and numerical variations. Our results indicate that normal spermatogenesis is accompanied by a significant incidence of germ cell chromosome abnormalities. The significance of these findings is discussed.

#### MATERIAL AND METHODS

Testicular biopsy material was obtained from seven healthy adult male volunteers, aged 26-47 years. The subjects were randomly selected from a larger group of inmate volunteers from the Washington State Penitentiary who were participating in a study of factors effecting spermatogenesis. All gave a history of previous fertility. Normal spermatogenesis was documented in each subject by repeated sperm counts over 56 million/cc (Gordon et al., 1967) normal urinary Follicle Stimulating Hormone (FSH) titers (Steelman & Pohley, 1953) and testicular histology. In addition, blood leukocyte (Moorhead et al. 1960) or short term testicular monolayer cultures (Paulsen, de Souza and Yoshizumi, 1964) for chromosome evaluation revealed normal male karyotypes in six of the subjects. The seventh subject (M-1) was unavailable for somatic cell karyotyping.

All biopsies were performed with local anesthesia. The tissue specimens, usually under 3 mm<sup>3</sup>, were immediately placed into hypotonic (0.7%) sodium citrate solution and processed by a modification of Evans et al (1964). Germ cells were teased from the tubules using fine forceps and left in hypotonic solution for 15 minutes at room temperature. Slides were prepared by flame-drying after 20 minutes fixation in acetic-methanol (1:3). An average of 10-20 slides were prepared from each subject. All slides were stained with tetrachrome and scanned under a Zeiss photomicroscope. Direct microscopic evaluation, as well as photographic analysis, was made on all appropriate cells which were intact. Disrupted cells were not analyzed for abnormalities. They were, however, tabulated as representing probable diploid cells in the determination of polyploid frequency.

#### RESULTS

A considerable number of well spread cells were present on most slides. The heteropycnotic sex vesicle could be detected at pachytene prophase, with the autosomal elements appearing as interwoven indistinguishable thin threads. Separate chromosome elements were seen in spermatogonia, primary, and secondary spermatocytes during cell division at metaphase.

The chromosomes at spermatogonial metaphase appeared as heavily condensed elements. Indistinct separation of the chromatid

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arms was characteristic of these chromosomes, in contrast to somatic cell mitotic preparations. Moreover, a definite centromere was frequently absent. Some elements did demonstrate a discrete central constriction, often accompanied by elongation of the area. Actual chromosome fragmentation was also seen (Fig. 20) in many cells. In spite of this frequent fragmentation, a modal number of 46 chromosome elements was found in all subjects (Table 12). Over 50% of the nonmodal cells exhibited evidence of chromosome loss, with modal counts under 46. These cells all appeared to be otherwise intact, and were interpreted as representing true loss of chromosomes.

Polyploid forms of spermatogonial metaphase were noted (Fig. 21) in six of the seven subjects. The other subject had only 5 cells present at spermatogonial metaphase and could not be adequately evaluated. Exact chromosome counts were often impossible in the polyploid cells, however most were tetraploid in nature. Occasional cells containing up to 120 chromosome elements were also noted. The incidence of polyploidy in these individuals ranged from 4.2 to 13.6% of the metaphase plates seen, with an average frequency of 7.4% (Table 13).

Bivalent association of homologous chromosomes was seen (Fig. 22) at diakinesis and first meiotic metaphase. The autosomal bivalents were usually united at 2 or 3 chiasma to form bivalents of varying configuration. Occasionally, up to 5 chiasma were apparent in one of the larger bivalents, while some of the smaller bivalents had only one chiasma. The sex chromosome bivalent was easily recognized in most cells by its characteristic linear configuration and terminal association. It was not possible to determine by light microscopy if the sex chromosomes were associated by a true chiasma or by other means of union. The XY bivalent was considered to possess a single true chiasma however, when the chiasma frequency per cell was determined.

Evaluation of cells which exhibited 23 distinct bivalents revealed an average of 50.1 chiasma per cell, with a range of 40-59 (Table 14). Since the number of chiasma per bivalent decreases in metaphase, the observed chiasma range may be partially secondary to inclusion of different stages of diakinesis and first meiotic metaphase.

Some cells failed to show bivalent association of all the chromosomes at first meiotic metaphase (Table 12). One of the smaller bivalents frequently appeared elongated, with actual separation of homologous chromosomes seen in 8.0% of the cells. Precocious separation of the sex chromosomes was also seen in all subjects, occurring in 4.5% of cells examined. There was



Fig. 20

Normal spermatogonial metaphase, exhibiting constrictions and tendency towards fragmentations

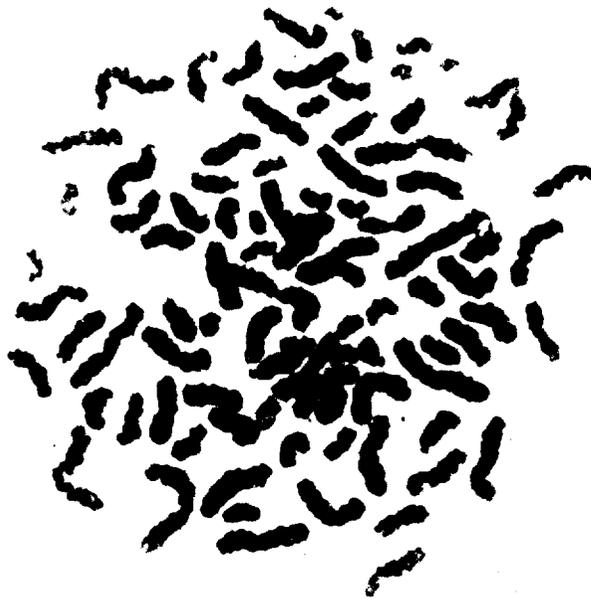


Fig. 21

Polyploid spermatogonial metaphase

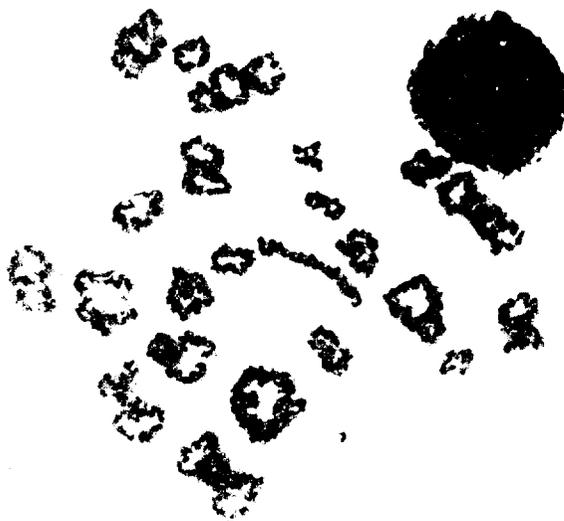


Fig. 22

Normal primary spermatocyte at diakinesis

TABLE 12

DISTRIBUTION OF CHROMOSOME COUNTS/CELL

Subject No.	Age	Sperm Count (M/cc)	Spermatogonial Metaphase		Diakinesis/First Meiotic Metaphase	
			< 46	46	23 bival.	22 bi & X+Y & 2 auto un.
M 1	28	69	1	3	15	1
M 7	29	172	3	14	24	2
M 9	32	127	11	22	43	3
M 14	42	75	2	18	6	1
M 15	34	161	2	6	44	4
M 17	32	80	19	40	49	5
M 21	49	85	5	17	38	2
Total Number of Cells Examined			40	120	219	18
				36		26

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TABLE 13

INCIDENCE OF POLYPLOID CELLS/SUBJECT

Subject No	<u>Spermatogonia Metaphase</u>		<u>First Meiotic Division</u>	
	Total Cells Present	No. Polyploid Cells (%)	Total Cells Present	No. Polyploid Cells (%)
M 1	5	0	23	0
M 7	47	2 (4.2)	53	1 (1.9)
M 9	79	6 (7.6)	95	1 (1.1)
M 14	32	2 (6.2)	17	1 (5.9)
M 15	22	3 (13.6)	94	6 (6.4)
M 17	111	9 (8.1)	71	2 (2.8)
M 21	41	3 (7.3)	56	1 (1.8)
<hr/>				
Total	337	25 (7.4)	409	12 (2.9)

1001951

TABLE 14

CHIASMA FREQUENCY PER CELL

Diakinesis & First Meiotic Metaphase

Subject No.	Chiasma/Cell	Range/Cell	Cells Counted
M 1	50.9	46 - 58	16
M 7	50.3	44 - 59	6
M 9	48.4	40 - 56	25
M 14	50.3	46 - 54	3
M 15	51.8	47 - 55	12
M 17	49.9	43 - 54	10
M 21	50.6	48 - 54	11

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1001952

no correlation between precocious separation of an autosomal bivalent with that of the sex chromosomes. Only one cell manifested simultaneous separation of both bivalents. The autosomal univalents always appeared to lie close together in the metaphase plate, whereas the sex chromosome univalents would often appear distant from each other. This would suggest, at least with the sex chromosomes, that the presence of univalents is not an artifact of technique, but a natural occurrence.

Polyploidy was also seen at diakinesis (Fig. 23) and first meiotic metaphase, with an average frequency of 2.9% of all cells (Table 13). Some cells appeared to represent polyploid forms, but had irregularity of the margin suggesting artifactual merging of two diploid cells. These latter cells were not considered to represent true polyploids, and were, therefore, tabulated as separate diploid forms.

Multivalent association was detected in 3 of the subjects (Fig. 24) in a total of six cells (1.5% incidence). Whereas 22 of the bivalents at diakinesis had normal configuration, one was noted to be abnormal. These abnormal elements appeared to represent trivalent associations, with an univalent arm connected by a single chiasma to the bivalent nucleus. It is also possible, however, that they reflected previous chromosome breakage with loss of large acentric fragments. Chromosome breakage and fragmentation were definitely seen in other cells, being found in all subjects. These cells appeared indistinct with evidence of degeneration and dissolution of nuclear material. Degenerating cells were evenly distributed on different slides, occurring with an average incidence of 4.4% of all cells (range 2.1-11%) (Fig. 25). They were not felt to be artifactual in origin, since surrounding cells maintained normal appearance and were free of distortion.

Haploid chromosomes of secondary spermatocytes were seen at metaphase, characterized by spiralization of their chromatid arms. Homologous chromatid arms were usually widely divergent from each other, often resulting in difficulty in actually identifying paired elements. It was not possible to quantitatively evaluate the chromosomes at this stage because of this technical difficulty. A few polyploid cells, however, were also seen at second meiotic metaphase.

#### DISCUSSION

These data clearly show that both numerical and structural abnormalities occur in the germ cell chromosomes in the presence of normal spermatogenesis. Germ cell mosaicism was present, as evidenced by the high incidence of nonmodal cells. The frequency of nonmodal cells in spermatogonia at metaphase was especially

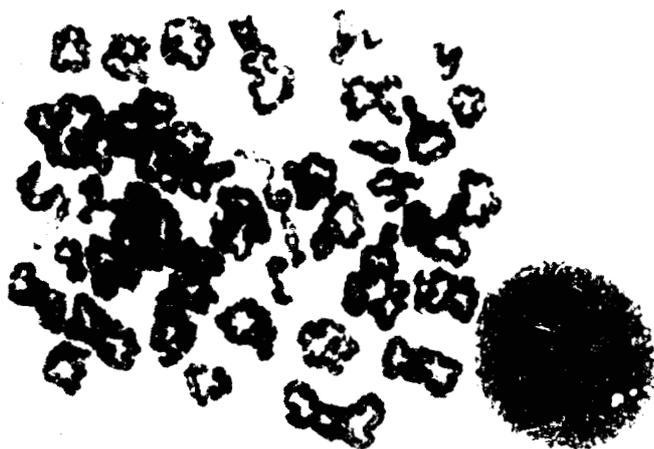


Fig. 23

Polyploid primary  
spermatocyte at  
diakinesis



Fig. 24

Multivalent association  
at diakinesis (arrow  
indicates trivalent)



Fig. 25

Degenerating cell at  
first meiotic metaphase

significant, Variability in number of spermatogonial chromosomes per cell has previously been observed in histologic testicular sections (Painter, 1923), suggesting that this phenomenon occurs in vivo. Several mechanisms may be involved in the production of these nonmodal cells. First, chromosome fragmentation may contribute to some of the cells which appeared to have greater than 46 chromosomes. The fragility of the spermatogonial metaphase chromosomes may be a reflection of artifactual damage due to technique of cell preparation, but may also occur in vivo. Loss of genetic material eventually results when fragmentation occurs in vivo, since the fragmented portion is unattached to a centromere for reduplication. Secondly, it is also possible that some of these cells represent true trisomies, with a net increase in DNA material. The presence of cells with multivalent association at diakinesis indicates that true hyperploidal cells are occasionally present in the germ cell stem-line.

Spermatogonia containing less than 46 chromosomes at metaphase cannot be explained by artifactual loss, since the cells all appeared to be otherwise intact. The presence of in vivo loss of chromosomes at spermatogonial metaphase is supported by finding less than 23 bivalents in some apparently intact cells at diakinesis and first meiotic metaphase. Hypomodal primary spermatocytes at diakinesis could result from artifactual disruption of the nuclear membranes and loss of one or more bivalents, however, one would expect to find these "lost" bivalents elsewhere on the slides. We have never seen isolated bivalent chromosomes on the slides, except in the immediate vicinity of obviously damaged cells. This is further evidence, therefore, that hypomodal cells at both spermatogonial metaphase and diakinesis probably occur in vivo, and are not a reflection of artifactual damage. It is of interest that similar hypomodal "apparently intact" cells at diakinesis were reported by Ford and Hamerton (1956), but attributed by those authors to be of no significance.

Polyploid germ cells were found at all three stages of cell division. The presence of polyploidy implies either a defect in the spindle mechanism or failure of cytoplasmic division following a normal mitosis. Defective cytoplasmic division would appear to be the most probable mechanism for the cells found, since multivalent associations were absent in the polyploid cells at diakinesis.

The decreasing frequency of polyploid cells seen in the more mature germ cells, suggests that some degeneration occurs. Actual degenerative changes were not noted in the polyploid cells however, in distinction to the findings of Darlington & Haque (1962). It is possible that these polyploid cells might be partly responsible for the high incidence of triploidy seen in spontaneous abortuses.

1001955

This is supported by the observation of an XYY sex chromosome complement in some triploid fetuses (Lanman, 1968), indicating that some must be related to paternal polyploidy.

Polyploid cells are not unique to the germinal cells. They have been occasionally seen in erythrocyte precursors in direct bone marrow squash preparations (Powsner & Berman, 1961), and are a normal characteristic of megakaryocytes.

The pairing of homologous chromosomes to form bivalents is the distinguishing feature of first meiotic prophase. These bivalents normally remain united during first meiotic metaphase, separating only during anaphase. Terminalization of chiasma may occur early during metaphase in some bivalents. Precocious separation of a bivalent was present in over 12% of cells at diakinesis and at the first meiotic metaphase. Previous authors have also noted the frequent appearance of precocious separation of either the sex chromosomes or an autosomal bivalent at this stage (Ford & Hamerson, 1956; Ferguson-Smith, 1961; Sasaki & Makino, 1965; Hulten et al., 1966). A major concern of all investigators, including the present authors, has been the inability to determine if these findings occur in vivo, or result from minor damage to cells during the preparation process.

Separation of a bivalent during metaphase may result in failure of the individual univalents to segregate on the equatorial plate. The clinical consequences would be similar to that of nondisjunction during anaphase, since both univalents may pass to the same spindle pole. Theoretically, three different types of secondary spermatocytes could be produced as a result of precocious separation of bivalents. Fifty per cent of these cells would be normal, or Type I; of the remainder, Type II secondary spermatocytes would contain both the X and Y chromosome while the Type III cells would contain only autosomes. Documentation of precocious separation of bivalents at first meiotic metaphase requires the observation of either Type II or III secondary spermatocytes. Unfortunately, the present techniques of chromosome preparation are not sufficiently developed to quantitatively evaluate the chromosome constitution of the secondary spermatocytes, and this documentation is lacking.

Our findings support the existence of an attrition rate during normal spermatogenesis. A major portion of the attrition process would appear to occur at the first meiotic division. Degeneration of each cell at the first meiotic division results in the loss of 4 potential spermatids. A decrease in spermatid formation averaging 17.6% would thus result from the degenerative cells observed at this stage alone.

The presence of documented chromosomal abnormalities during normal spermatogenesis may be a significant factor in the observed

1001956

attrition of germ cells. Either an excess or a deficiency of essential chromatod material may contribute to defective pairing of chromosomes at the first meiotic prophase, with resulting degeneration. The intratesticular degeneration of genetically unbalanced germ cells may be considered to represent a beneficial effect in normal men, by decreasing the incidence of defective gametes. Impairment of this protective mechanism in some normospermic males could contribute to cases of "idiopathic infertility" clinically observed.

## 2. Preliminary Post-Irradiation Studies

With our study in normal non-irradiated subjects as a baseline we have started to investigate the germ cell chromosomal pattern in our volunteers following irradiation. At this time we have preliminary observations on two such subjects. Table 15 lists the information obtained so far.

RV-8 received 50 r four years prior to obtaining the biopsy specimen. There was no evidence of abnormal configurations at diakinesis. Statistical evaluation of the incidence of polyploid cells was not possible due to the small number of cells available for examination. There was no increase in the incidence of random bivalent loss per cell as compared to the control population. Therefore, with this specimen we were unable to detect any abnormalities that could be attributed to his exposure to irradiation (Table 15).

RV-14 received 400 r four years prior to securing his testicular biopsy specimen for examination of germ cell chromosomes. There was no increased incidence of precocious separation of the (Fig. 26) "sex" bivalents nor autosomal bivalents. There was a slightly higher incidence (10%) of random bivalent loss per cell. The frequency of polyploid and degenerating cells is similar to that encountered in our control population (Table 15).

Two cells are of particular interest. One well-spread cell lacks the sex chromosomes. The other cell appears to have a multivalent sex chromosome which suggests the presence of an XXY configuration. (Figures 27 and 28)

In summary, the presence of seven cells lacking 1-3 of the bivalents plus the cell exhibiting a multivalent sex chromosome association suggests that the spindle membrane (spermatogonial metaphase) may have been disrupted with a resultant alteration in chromosome pairing. Further studies will be required to define the role of irradiation in creating these abnormalities

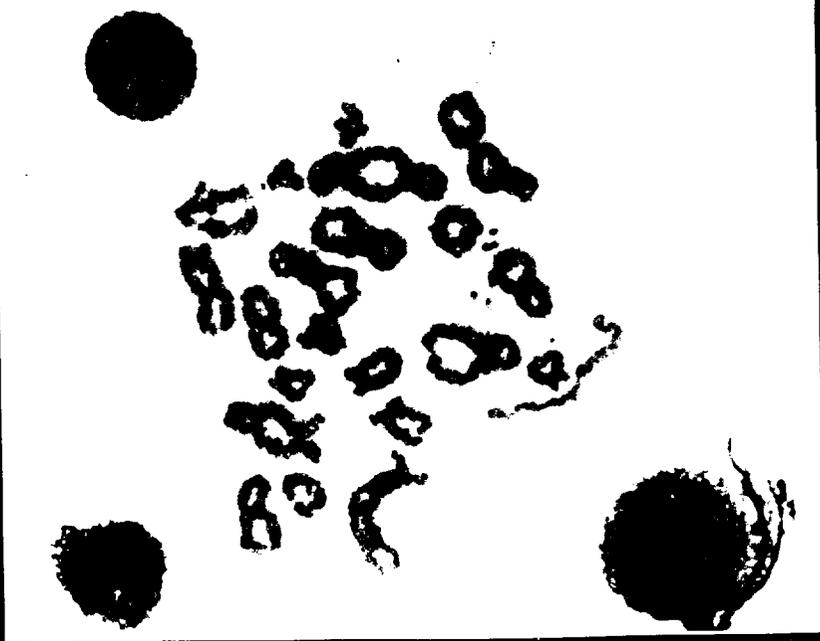


Figure 26.

RV-14 Normal Diakinesis  
four years following  
400 r.

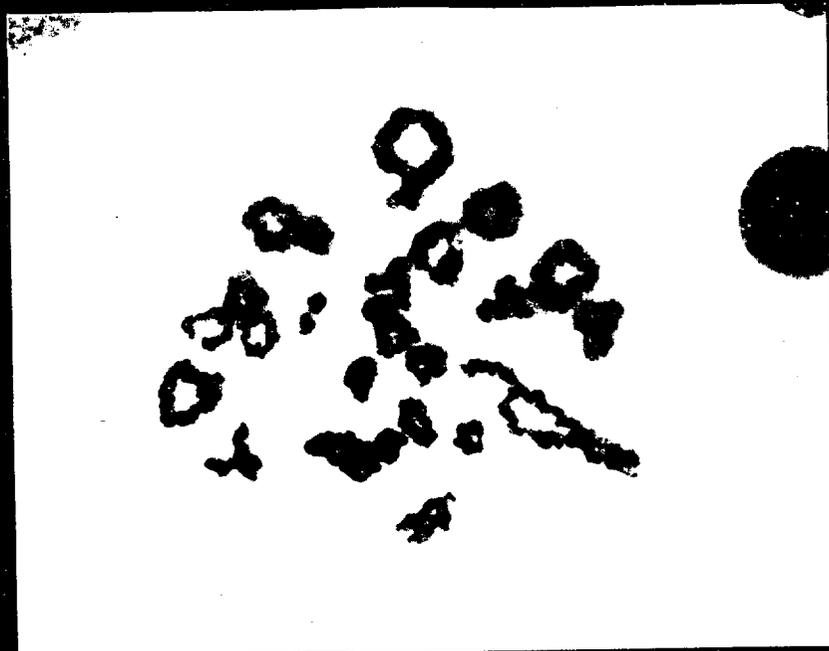


Figure 27.

RV-14 Diakinesis  
lacking the multivalent  
sex chromosome. Four  
years following 400 r.



Figure 28.

RV-14 Diakinesis with  
multivalent sex  
chromosome, suggesting  
XXY configuration.  
Four years after 400 r.

TABLE 15

TESTICULAR GERM-CELL ANALYSIS OF  
MEIOTIC CHROMOSOMES FOLLOWING IRRADIATION

RV-8 50 r Four Years Earlier

normal diakinesis (23XY).....	23 cells
normal XY, but indefinite count**.....	10 cells
Precocious separation of bivalent.....	0 cells
Missing one bivalent, (22XY)*.....	1 cell
Missing two bivalents (21XY)*.....	1 cell
Polyploid cell.....	<u>3 cells</u>
Total examined	38 cells

RV-14 400 r Four Years Earlier

normal diakinesis (23 XY).....	26 cells
normal XY, indefinite count**.....	19 cells
precocious separation of bivalent	
sex chromosomes (X+Y).....	4 cells
autosome.....	1 cell
Missing one bivalent (22XY)*.....	3 cells
Missing three bivalents (20XY)*.....	3 cells
Polyploid cell.....	2 cells
Degenerating cell.....	1 cell
Possible XXY association.....	1 cell
Missing sex chromosomes (22+0).....	<u>1 cell</u>
Total examined	61 cells

\* Cell appears to be otherwise intact

\*\* Bivalent overlapping present, unable to give exact count

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V. Changes in Urinary Gonadotrophin Excretion Titters Following Irradiation

It is well-known that a reciprocal relationship exists between gonadal function and urinary gonadotrophin levels. However, the precise details and mechanisms of this relationship are not clearly understood. When sufficient primary testicular damage occurs urinary gonadotrophin levels become elevated. This increase is theoretically proportional to the degree of gonadal damage and is not an all or none type of response.

Measurement of serial gonadotrophin titters were performed before and following irradiation: (1) to determine the radiation dosage necessary to produce elevated gonadotrophins, (2) to determine the time lapse following irradiation before elevated gonadotrophin titters occur, (3) to relate the degree and if possible the duration of gonadotrophin elevation to radiation dosage and (4) to determine which of the gonadotrophins (or if both) FSH and/or ICSH (LH) were responsible.

Our preliminary data indicated that urinary ICSH (LH) activity as determined by bioassay was not significantly increased by the radiation dosage (7.5-400 r) employed in our study. These observations have been extended and are discussed elsewhere in this report. Because of these observations and because we wanted to evaluate the impact of disturbed spermatogenesis on gonadotrophin excretion, the Steelman-Pohley bioassay method which measures primarily FSH activity has been used instead of the general gonadotrophin assay (GGA) to monitor gonadotrophin levels since 1965.

In view of the rather wide confidence limits for the results obtained by bioassay the criteria for concluding that significant increases in gonadotrophin titters had occurred were established as follows:

1. The mean level for any post-irradiation titer had to exceed 2 S.D. of the control population's weighted mean titer.
2. The mean level for any post-irradiation titer of any given individual had to exceed 2 S.D. of his own control gonadotrophin level. This criterion was necessary since a few individuals exhibited control gonadotrophin titters which were above our normal population's control gonadotrophin titer.
3. The mean levels of two successive post-irradiation gonadotrophin assay titters had to fulfill both of the above criteria before an individual was considered to have demonstrated a significant elevation in urinary gonadotrophin excretion.

Data with respect to urinary FSH and GGA titers from all subjects receiving 7.5-400 r are presented in the following paragraphs. Individuals with insufficient data are excluded from this report.

A. 400 r (Table 16)

Six men were studied at this dose level. Two were evaluated by serial urinary FSH titers. Each demonstrated elevated titers by 26 and 46 days post-irradiation, respectively. Of the four studied by GGA assays, three exhibited significant elevation in titers within 60 days while the fourth demonstrated increased GGA titers somewhere between 46 and 103 days post-irradiation.

Once elevated the FSH and GGA titers remained increased for the duration of our observation period. In two subjects followed for the longest period our observation period extended to 452 and 489 days post-irradiation. It would appear then that elevated gonadotrophin titers would probably exceed 500 days post-irradiation when the individual was exposed to 400 r.

B. 100 r (Table 17)

Twenty-five volunteers have received 100 r to date. Eighteen of this group have been followed for a sufficient period of time to allow interpretation of their gonadotrophin titers. Thirteen men demonstrated elevated FSH titers while five exhibited increased GGA titers. FSH titers became elevated somewhere between 13 and 121 days post-irradiation.

Although many of the volunteers left the program before their gonadotrophin titers returned to pre-irradiation levels, in those men who could be followed long enough control gonadotrophin levels were achieved between 202 and 642 days post-irradiation

With respect to GGA titers four of five men studied demonstrated a rise in titer by 30 to 120 days which remained elevated for 248 to 440 days post-irradiation.

The single individual who did not exhibit increased GGA titers was studied for 429 days. His seminal fluid became azoospermic at 131 days. His urine collections were reliable and he remained in good general health. One reason for his failure to show a significant increase in gonadotrophin excretion in the face of azoospermia is not apparent.

C. 50 r (Table 18)

FSH titers were measured serially in five men who received 50 r to their testes; three men who were irradiated earlier in our

1001963

TABLE 16: FSH Titer\* Following 400 r

400 r RV No.	Last Normal FSH Titer (days)	Initial Elevation FSH Titers (days)	Peak Elevation (days)	Elevated FSH Titer (days)	Last Elevated FSH Titer (days)	Highest Tite I.U./24 hrs
22	25	46	145	>294	>294	64.4
57	12	26	68	> 68	> 68	35.8
<hr/>						
General Gonadotrophin Levels						
5		57	90	125 +	125 +	6.5
14	28	56	229	489 +	489 +	5.6
23		35	200	452 +	452 +	7.8
26	46	103	190	256	256	5.3

\* The numbers under these columns indicate the days following x-ray exposure.

1001964

TABLE 17: FSH Titer\* Following 100 r

100 r RV No.	Last Normal FSH Titer (days)	Initial Elevation FSH Titers (days)	Peak Elevation (days)	Elevated FSH Titer (days)	Last Elevated FSH Titer (days)	Highest Titer I.U./24 hrs.
2		31	75	133	133	67.6
7		31	121	121	121	42.1
28		65	107	221	221	121.3
32	65	121	121	184	184	45.6
38	74	94	94	129	129	20.0
42		13	195	265	265	74.8
43	31	47	274	435	435	73.5
47	50	63	199	199	199	47.6
48		47	325	521	521	106.9
71	71	83	83	339	339	21.7
74	66	79	160	175	175	52.8
79		48	285	298	298	96.1
120	10	31	287	537	537	52.7
General Gonadotrophin Levels						
3	429					mg-eq UPM-1/ 24 hrs
4	20	68	115	297	297	4.8
6	90	120	252	432	432	7.3
16	28	30	58	248	248	12.8
27	56	96	108	440	440	7.3

\* The numbers under these columns indicate the days following x-ray exposure.

1001965

TABLE 18: FSH Titer\* Following 50 r

50 r RV No.	Last Normal FSH Titer (days)	Initial Elevation FSH Titers (days)	Peak Elevation (days)	Elevated FSH Titer (days)	Last Elevated FSH Titer (days)	Highest Titer I.U./24 hrs.
78	12	54	115	273	273	56
127	68	82	188	202	202	45.2
133	61	103	103	174	174	53.4
144	117	131	174	174	174	22.9
147	47	61	145	145	145	68.3
General Gonadotrophin Levels						
8	78	106	302	302	302	4.9
15	35	42	91	91	126	5.2
17	425					

\* The numbers under these columns indicate the days following x-ray exposure.

1001966

study were examined by GGA assays. In the group studied with FSH assays all showed an increase by 54 to 131 days post-irradiation. Control FSH titers appeared by the 216 and 301st day post-irradiation in the two men followed the longest period of time. The remaining three men of this group (FSH assays) have maintained elevated titers up to the present time which is 174 days post-irradiation.

Two of the three inmates who were followed by GGA assays demonstrated a rise in titer by 42 and 106 days, respectively. The other subject did not exhibit an increase in gonadotrophin excretion despite the development of azoospermia. This observation again points to the fact that the GGA assay is not as sensitive an indicator of damaged spermatogenesis as the FSH assay is.

D. 30 r (Table 19)

At this dosage two volunteers were studied by FSH assays and two by GGA assays. The two men followed by FSH assays demonstrated a significant rise in titers by 122 and 124 days and maintained this increase for a period of time in excess of 213 and 251 days post-irradiation, respectively. In the "GGA group" there was no significant elevation in titers by 156 and 197 days post-irradiation.

E. 15 r (Table 20)

A total of eight men received 15 r; six were followed by FSH assays and two with GGA. In the "FSH group" one subject demonstrated a rise in titer by 121 days with a return to control titers by 212 days post-irradiation. The remaining men in this group were followed for periods of time varying between 163 to 672 days post-irradiation without exhibiting a significant rise in FSH titers.

In the "GGA group" one subject showed a rise in titer by 51 days which remained elevated throughout the 337 days of observation post-irradiation. The other subject did not show an increased titer during the 142 days he was studied.

F. 7.5 r (Table 21)

All six volunteers who received this dosage were studied with FSH assays. None of this group demonstrated an increase in FSH titers which fulfilled our previously mentioned criteria.

G. Summary

In Table 22 the cumulative data with regard to urinary FSH titers are presented. The data are arranged according to the radiation

TABLE 19: FSH Titer\* Following 30 r

RV No.	Last Normal FSH Titer (days)	Initial Elevation FSH Titters (days)	Peak Elevation (days)	Elevated FSH Titer (days)	Last Elevated FSH Titer (days)	Highest Titer I.U./24 hrs.
29	81.	124	151	>251		62.2
34	80	122	150	>213		71.5
101	Off program 1 month post-irradiation					
<u>General Gonadotrophin Levels</u>						
21	156 +					mg-eq UPM-1/24 hrs.
30	197 +					

\* The numbers under these columns indicate the days following x-ray exposure.

1001968

TABLE 20: FSH Titers\* Following 15 r

15 r RV No.	Last Normal FSH Titer (days)	Initial Elevation FSH Titers (days)	Peak Elevation (days)	Last Elevated FSH Titer (days)	Highest Titer I.U./24 hrs.
31	672				
39	172				
41	79	121	149	178	30.8
72	176				
154	163				
163	174				
<u>General Gonadotrophin Level</u>					
18	142				
20		51	207	337	8.0

mg-eq UPM-1/  
24 hrs.

\* The numbers under these columns indicate the days following x-ray exposure.

1001969

TABLE 21: FSH Titters\* Following 7.5 r

7.5 r RV No.	Last Normal FSH Titters (days)	Initial Elevation FSH Titters (days)	Peak Elevation (days)	Last Elevated FSH Titer (days)	Highest Titer I.U./24 hrs.
10	400				
13	296				
76	171				
99	156				
119	265				
141	175				

\* The numbers under these columns indicate the days following x-ray exposure.

1001970

TABLE 22: Urinary FSH Titers

<u>Dose x-ray</u> (r)	<u>30</u>	<u>60</u>	<u>90</u>	<u>120</u>	<u>150</u>
400	1/2	2/2	2/2	2/2	2/2
100	4/13	9/13	12/13	13/13	13/13
50	0/5	2/5	3/5	4/5	5/5
30	0/2	0/2	0/2	2/2	2/2
15	0/6	0/6	0/6	1/6	1/6
7.5	0/6	0/6	0/6	0/6	0/6

1001971

dose received and duration in days post-irradiation. The denominators indicate the number of subjects who were irradiated while the numerators indicate the number of men who demonstrated elevated FSH titers by that point in time. The time was not extended beyond 150 days since none of the men developed an initial elevation in FSH titer later than that time.

The time lapse in "post-irradiation days" for increased FSH titers to appear is roughly inversely proportional to the x-ray dose received.

With regard to the duration of elevated FSH titers no definitive statement can be made at this time. However, it also appears to be dose-related since at 100 r FSH titers were elevated longer than at 50 r or 30 r.

#### H. Conclusions

1. 15 r is the lowest radiation dose tested that produced enough damage to result in elevated gonadotrophins.
2. The time lapse following irradiation for gonadotrophin elevation to occur is related to radiation dosage i.e. the higher the dose the earlier the elevation in gonadotrophin occurs.
3. The duration of gonadotrophin elevation is related to the radiation dose i.e. the higher the dose the longer the gonadotrophins remained elevated.
4. Specific FSH titers were more often elevated than GGA at the lower doses where germinal epithelial destruction was less marked and, therefore, are the more sensitive indicator of spermatogenetic function.
5. The time lapse following irradiation for gonadotrophin elevation to occur is correlated with the time lapse for a decrease in sperm count to occur.

1001972

VI. Biologic Effects of X-ray Irradiation on Leydig-Cell Function

Animal data suggests that the Leydig cells of the testis are considerably radio-resistant. In our study we have been evaluating Leydig cell function to determine whether or not human Leydig cell function remains unaltered when the testes are exposed to doses of x-ray which clearly results in denuding the germinal epithelium and azoospermia.

With the collaboration of Dr. Hortense Gandy at Cornell Medical Center, plasma testosterone (T) has been measured by a double isotope derivative technique. Preliminary data which have been submitted in previous progress reports showed that both a significant diurnal and daily variation in plasma T existed prior to irradiation. Despite this variation plasma T levels would still directly reflect Leydig-cell function and testosterone secretion rate since, in the male, testosterone emanates primarily from the Leydig cells. Furthermore, it is unlikely that there would be any changes in testosterone clearance rate as a consequence of local testicular irradiation.

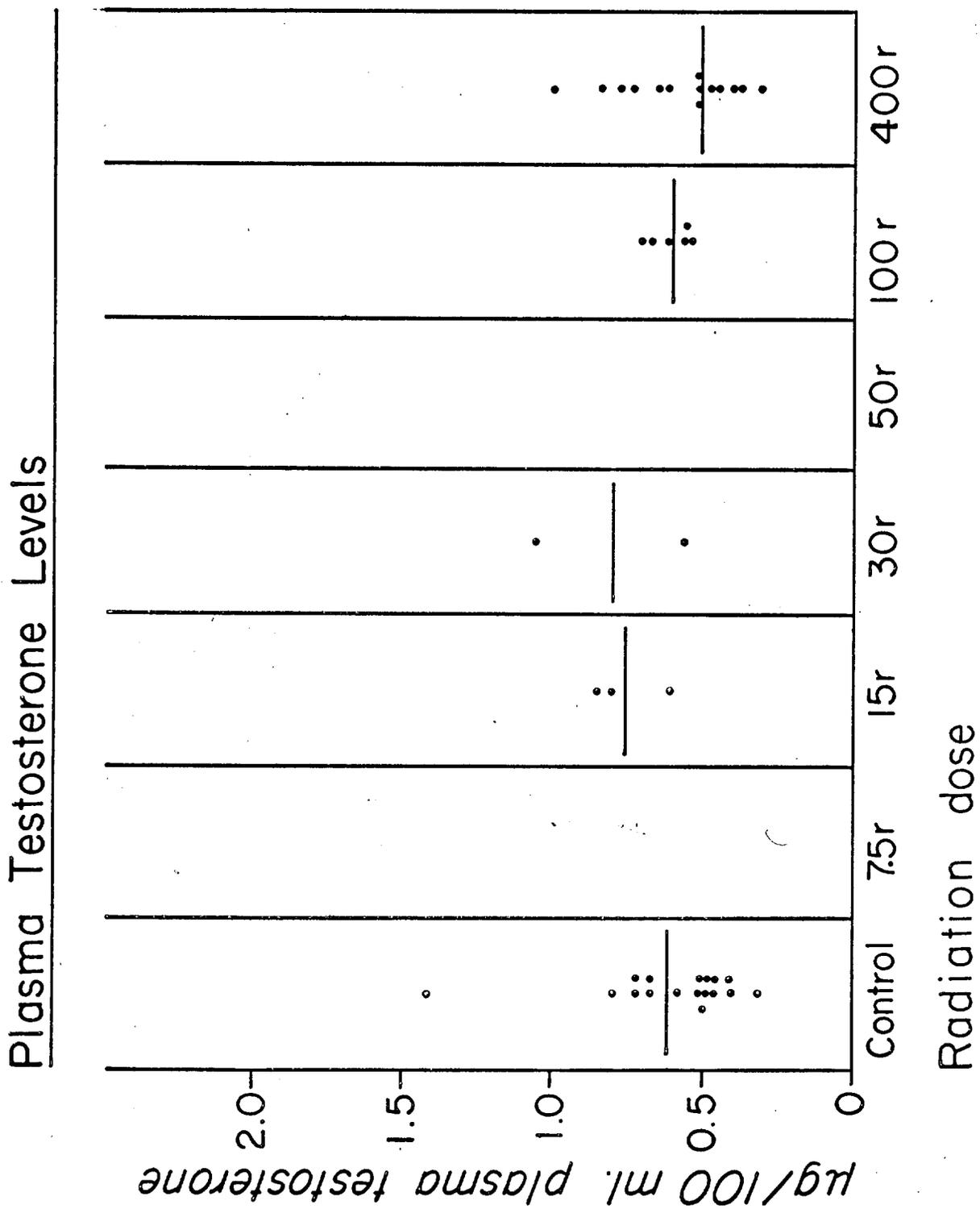
Testicular function has been studied following exposure to x-ray doses of 7.5 to 400 r. In seven men plasma T levels were measured at intervals of 4 and 72 hours and 2 weeks post-irradiation. No significant decrease in plasma T titers was detected even at the 400 r dose level (Table 23). All values, including those obtained during the control period, are shown in a scattergram for graphic illustration (Fig. 29). Also see Tables 24 through 28.

Since a temporary decrease in Leydig-cell function could have been compensated for by an increase in pituitary ICSH (LH) release with subsequent return of normal testosterone production, serum and urine LH titers were examined following irradiation.

Early in our study urinary LH excretion titers were measured by the hypophysectomized male rat ventral prostate method. The gonadotrophic potency of urinary extracts was computed by parallel line analysis in terms of the second international reference preparation. For our normal male population during the control period the mean urinary LH level was 6.8 I.U./24 hours. The upper limit (95% confidence limit) was 19.8 I.U./24 hours. Following irradiation fourteen men were studied at monthly intervals for up to 14 months. No increase in urinary LH excretion could be detected (Table 24 and Figure 30). Since the dose response slope for the bioassay of LH is rather flat, i.e.  $b = \text{circa } 20$ , our index of discrimination is such that small changes in LH levels might not be detected.

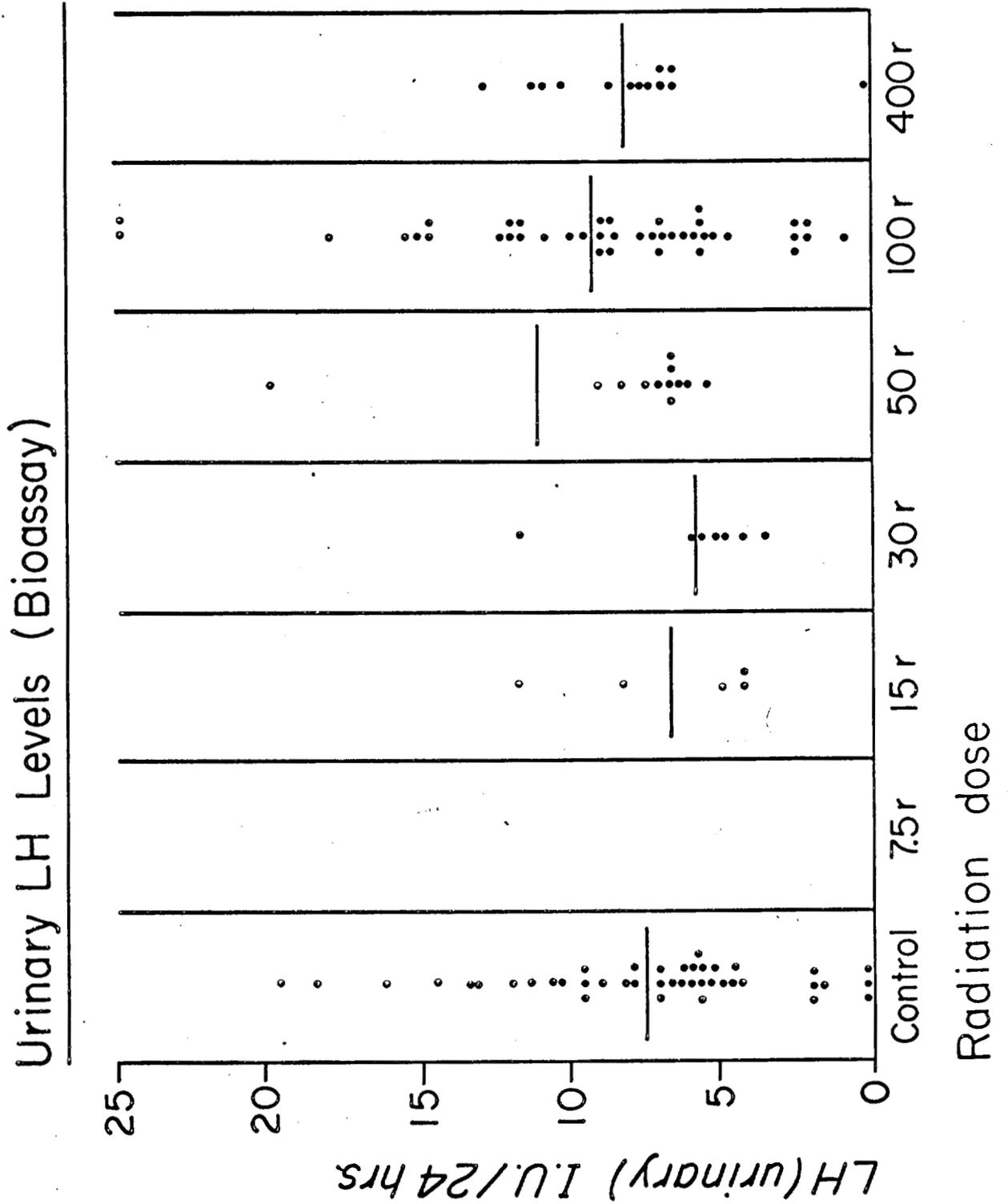
1001973

Figure 29. PLASMA TESTOSTERONE LEVELS



1001974

Figure 30. URINARY LH LEVELS



1001975

Table 23: Leydig-Cell Function Studies Following 400 r

CONTROL POST IRRADIATION

RV	LH	Specimen Date	Plasma T µg%	Specimen Date	Date of Radiation	LH	Specimen Date	Days	Plasma T µg%	Specimen Date	Day
5	9.72*	8/24/64	0.80	5/3/65	5/3/65				0.74	5/3/65	4 h
						12.99*	6/28/65		0.84	5/6/65	72 "
									1.0	5/17/65	4 w
14	None				6/24/64	11.43*	7/20/64	1 mo.			
						7.8 "	8/17/64	2 mos.			
						7.5 "	9.28/64	3 mos.			
						10.98"	1/4/65				
						8.07"	2/9/65				
						6.7 "	6/8/65	12 mos.			
22	5.99*	10/19/64			2/19/65	None			0.52	2/19/65	4 h
									0.51	2/22/65	72
									0.46	3/5/65	2 w
									0.50	4/14/65	7½
23	< 0.2*	3/11/64	0.72	6/24/64	6/24/64				0.35	6/24/64	4 h
	< 0.2"	3/14/64							0.47	6/25/64	24
									0.40	6/27/64	72
						0.2*	7/27/64		0.30	6/5/64	
						7.04"	8/10/64	2 mos.	0.76	8/3/64	
									0.61	9/23/64	
						8.78*	10/5/65	3½ mos	0.64	10/28/64	
						11.0 "	1/12/65	7 mos.	0.38	1/20/65	
						7.13"	4/19/65	10 mos			

100197b

100197c

POST IRRADIATION

CONTROL

RV	LH	Specimen		Date of Radiation	LH	Specimen Date	Days	Plasma T µg%	Specimen Date	Days	Plasma T µg%	Specimen Date	Day
		Date	T µg%										
26	< 2*	3/10/64		6/24/64	10.4*	1/12/65	7 mos						
	4.65*	3/26/64			6.7"	2/15/65	8 mos						
	8.14"	3/30/64											
63	4.7*	4/11/67	0.32	5/24/67	None								
	***83	19.7**	4/19/66	5/8/68	24.8**	8/17/68	3 mos.						
		19.7 "	1/18/68	0.50		20.0 "	8/31/68						
	25.3 "	1/31/68			14.6 "	9/14/68							
					18.9 "	9/28/68	4½ mos.						

\* I.U./24 hrs.  
 \*\* mI.U./ml  
 \*\*\* Sertoli Cell Syndrome

1001977

Table 24: Leydig-Cell Function Studies Following 100 r

RV	CONTROL			POST IRRADIATION						
	LH	Specimen Date	Plasma T µg%	Date of Radiation	LH	Specimen Date	Day	Plasma T µg%	Specimen Date	Day
2	7.04*	10/26/64			None					
3	<0.2 "	6/29/64		9/9/64	9.2*	9/14/64	2 mos.			
	<0.2 "	8/24/64			0.09"	11/16/64	3 mos.			
					< 2.2 "	12/7/64	4 mos.			
					2.7 "	1/4/65	5 mos.			
					12.4 "	2/8/65	6 mos.			
					< 2.5 "	3/8/65	7 mos.			
					< 2.5 "	4/5/65	8 mos.			
					< 2.2 "	5/5/65	9 mos.			
					6.39"	6/7/65				
4	None			9/9/64	10.95*	9/28/64	2 mos.			
					9.6 "	11/16/64	3 mos.			
					18.0 "	12/8/64	5 mos.			
					11.9 "	2/9/65	6 mos.			
					7.12"	3/8/65	9½ mos.			
					7.8 "	6/28/65				
6	16.39*	4/17/64		4/22/64	31.29*	5/3/64	2 mos.			
					10.06"	6/4/64	5 mos.			
					15.51"	6/18/64	7½ mos.			
					8.6 "	6/20/64	8½ mos.			
					9.01"	9/28/64				
					12.02"	12/7/64				
					14.8 "	1/4/65				
7	11.52*	6/29/64		5/14/66	None					
	6.69"	8/19/64								

1001978

CONTROL

POST IRRADIATION

RV	LH	Specimen Date	Plasma T µg%	Specimen Date	Date of Radiation	LH	Specimen Date	Day	Plasma T µg%	Specimen Date	Day
16	9.72*	3/26/64			4/7/64	12.23*	4/16/64	1 mo.			
						5.6 "	5/3/64	2 mos.			
						7.35"	6/4/64				
						9.0 "	6/6/64				
						5.75"	6/16/64				
						6.7 "	6/20/64				
						5.4 "	8/24/64				
						18.2 "	10/5/64	6 mos.			
						15.09"	12/7/64	8 mos.			
						9.0 "	1/11/65	9 mos.			
						7.6 "	6/29/65	14 mos.			

(Table 24 Cont'd)

19	13.5*	2/17/64			9.22.65	None					
	5.32"	6/8/64									
	13.3"	6/14/64									
	< 2.0"	7/20/64									
	9.09"	8/24/64									
27	< 2.0"	2/17/64			4/22/64	5.57*	5/7/64				
	> 7.36"	4/13/64				14.86"	6/6/64				
	5.9 "	4/20/64				5.75"	6/16/64	2 mos.			
						8.8"	7/27/64	3 mos.			
						11.92"	8/10/64				
						> 7.2"	10/12/64	6 mos.			
						8.7"	1/11/65	9 mos.			
						5.9"	4/19/65	12 mos.			
28	6.3*	8/17/64			5/3/65	< 4.8*	7/6/65	2 mos.			
	19.8"	10/26/64				25.88"	8/16/65				
32			0.47	5/3/65					0.53	5/3/65	4 hr
									0.67	5/6/65	72 "
									0.70	5/17/65	2 w
						3.6**	7/26/67				

1001979

POST IRRADIATION

CONTROL

RV	LH	Specimen Date	Plasma T $\mu$ g%	Specimen Date	Date of Radiation	LH	Specimen Date	Plasma T $\mu$ g%	Specimen Date	Day
38		5/3/65	0.52	5/3/65	5/3/65			0.62	5/3/65	4 hr
42	10.59*	4/15/64			5/18/66	None		0.55	5/6/65	72 hr
53	< 2.85"	10/25/64	0.41	10/28/64	9/22/65	None		0.55	5/17/65	2 wks
149	None				9/11/68	11.4**	10/27/68			
						10.2 "	11/9/68			2 mos.
						14.5 "	11/23/68			
						16/0 "	12/7/68			3 mos.

\* I.U./24 hrs.  
 \*\* mI.U./ml

1001980

Table 25: Leydig-Cell Function Studies Following 50 r

CONTROL				POST IRRADIATION					
RV	LH	Specimen Date	Plasma T µg%	Specimen Date	Date of Radiation	LH	Specimen Date	Day	
8	5.70*	4/17/64			11/20/64	6.75*	12/7/64		
	6.87"	5/5/64				7.51"	3/8/65		
						5.97"	5/3/65	7 mos.	
						6.23"	6/12/65		
15	14.62*	9/6/64			9/9/64	8.4*	9/28/64		
						6.42"	10/14/64		
						6.6"	11/16/64		
						20 "	12/8/64		
						7.26"	6/28/65	9½ mos.	
17	10.59*	7/27/64			9/9/64	5.55*	10/5/64		
						9.24"	12/7/64		
						1.46"	3/8/65		
						6.6 "	5/6/65		
						6.5 "	6/7/65	9 mos.	
78			0.47	6/3/65	11/30/67	8.0**	12/6/67		
			0.41	3/28/66		4.8 "	12/20/67		
		6.4**	3/28/66	0.68		3/29/66	6.0 "	1/3/68	
				0.48		3/30/66	15.6 "	1/17/68	
		10.6 "	3/30/66	0.48		4/11/66	14.7 "	1/31/68	
		5.9 "	4/11/66	0.48		4/25/66	13.5 "	2/14/68	
		8.6 "	4/25/66	0.68			12.6 "	2/28/68	
							20.0 "	3/13/68	
							11.5 "	3/27/68	
							26.0 "	4/10/68	
					17.8 "	4/24/68			
					21.5 "	5/8/68			
					14.7 "	5/22/68			
					17.0 "	6/5/68			

1001981

(Table 25 Cont'd)

RV	Specimen		Plasma Specimen		Date of Radiation		LH		Specimen	
	LH	Date	T	ug%	Radiation	LH	Date	Day		
127	8.4**	3/15/67			5/8/68	16.5**	7/17/68	11 day		
	10.5 "	3/22/67				12.0 "	7/17/68	1 mo.		
	15.2 "	2/28/68				19.0 "	6/19/68	2 mos.		
	11.0 "	3/3/68				19.0 "	7/3/68			
	10.5 "	3/27/68				14.5 "	7/17/68	3 mos.		
	15.4 "	4/10/68				14.5 "	7/31/68			
	11.7 "	4/27/68				15.3 "	8/14/68			
	11.8 "	5/8/68				17.4 "	9/28/68	4½ mos.		
	133						6/19/68	20.6**	8/17/68	2 mos.
					14.7 "	8/31/68		3 mos.		
					13.1 "	9/14/68				
					11.5 "	9/28/68		4 mos.		
					14.5 "	10/12/68				
					11.8 "	10/27/68				
					10.0 "	11/9/68		5 mos.		
					14.0 "	12/7/68		6 mos.		
144						6/19/68		16.0**	8/17/68	2 mos.
					7.0 "		8/31/68	3 mos.		
					24.0 "		9/14/68			
					14.4 "		9/28/68	4 mos.		
					7.4 "		10/12/68			
					10.8 "		10/27/68			
					12.6 "		11/9/68	5 mos.		
					11.5 "		11/23/68			

1001982

(Table 25 Cont'd)

CONTROL				POST IRRADIATION			
RV	LH	Specimen Date	Plasma T µg%	Specimen Date	LH	Date of Radiation	Day
147				8/17/68	15.1**	6/19/68	2 mos.
				8/31/68	22.8 "		
				9/14/68	16.6 "		3 mos.
				9/28/68	18.6 "		
				10/12/68	19.4 "		
				10/27/68	16.0 "		4 mos.
				11/9/68	15.0 "		
				11/23/68	13.8 "		5 mos.
				12/7/68	15.7 "		

\* I.U./24 hrs.  
 \*\* mI.U./ml

1001983

Table 26: Leydig-cell Function Studies Following 30 r

RV	CONTROL				POST IRRADIATION				
	LH	Specimen Date	Plasma T µg%	Specimen Date	Date of Radiation	LH	Specimen Date	Plasma T µg%	Specimen Date
21	9.16*	10/21/64			11/20/64	< 5*	1/11/65		
	5.99"	11/16/64							
30	4.42*	3/9/64			11/20/64	< 60*	1/4/65	1.06	11/20/64
	5.7 "	3/25/64				< 4.4"	2/15/65	0.56	11/23/64
	5.7"	3/30/64	1.44	11/20/64		3.6"	3/15/65		
						5.2"	4/26/65		
						5.7"	5/17/65		
						<11.92	6/7/65		
34	10.5*	4/14/64				None			
	12.13"	10/14/64			2/19/65				
	5.0"	10/21/64							
101	17.0**	7/26/67	0.52	3/28/67	11/30/67	14.0**	12/6/67		
	9.6"	11/30/67	0.59	3/29/67		16.0"	1/3/68		

\* I.U./24 hrs.  
 \*\* mI.U./ml

1001984

Table 27: Leydig-Cell Function Studies Following 15 r

POST RADIATION

CONTROL

RV	LH	Specimen		Date of Radiation	LH	Specimen Date	Day	Plasma T $\mu$ g%	Specimen Date	Day	Plasma T $\mu$ g%	Specimen Date	Day
		Date	T $\mu$ g%										
18	None			11/20/64	4.03*	12/7/64							
20	< 2*			11/20/64	>8.4*	1/11/65	2 mos.						
	18.6*	10/26/64			<4.43"	3/8/65	4 mos.						
					5.06"	4/5/65	5 mos.						
					<11.92"	6/14/65	7 mos.						
39	7.44**	11/13/67		11/30/67	9.9**	12/6/67	1 mo.						
					13.1 "	12/20/67							
					7.7 "	1/3/68							
					10.0 "	1/17/68	2 mos.						
					10.4 "	1/31/68							
					13.7 "	2/14/68	3 mos.						
					13.5 "	2/28/68							
					10.2 "	3/13/68							
					12.2 "	4/10/68	5 mos.						
					9.8 "	4/24/68							
					9.1 "	5/8/68							
					12.0 "	5/19/68	6 mos.						
					10.4 "	6/5/68							
					9.2 "	7/3/68	7 1/2 mos.						
					11.6 "	7/17/68							
41								0.71	2/19/65		0.62	2/19/65	4 hr
											0.81	2/22/65	72 "
											0.86	3/5/65	2 wk
72	None			6/19/68	14.8**	8/17/68	2 mos.						
					18.0 "	8/31/68							
					14.5 "	9/14/68	3 mos.						
					17.8 "	9/28/68							
					20.4 "	10/1/68	4 1/2 mos.						
					18.8 "	10/27/68							
154	None			6/19/68	7.6**	8/17/68	2 mos.						
					13.6 "	8/31/68							
					11.2 "	9/14/68	3 mos.						

1001985

RV	LH	Specimen		Date of Radiation	LH	Specimen		Day	Plasma T $\mu$ g%	Specimen	
		Date	T $\mu$ g%			Date	Date			Date	Day
163			11.0**			9/28/68					
			16.3 "			10/12/68		4 mos.			
			12.2 "			10/27/68					
			14.4 "			11/9/68		5 mos.			
			16.6 "			11/23/68					
			7.9**		6/19/68		8/17/68		2 mos.		
			16.8 "			8/31/68					
			15.0 "			9/14/68		3 mos.			
			15.0 "			9/28/68					
			12.5 "			10/12/68		4 mos.			
			8.8 "			10/27/68					
			16.6 "			11/9/68					
			9.7 "			11/23/68		5 mos.			
			10.4 "			12/7/68					

\* I.U./24 hrs.  
 \*\* mI.U./ml

100198b

CONTROL			POST IRRADIATION			
RV	LH	Specimen Date	Date of Radiation	LH	Specimen Date	Day
10	5.55*	11//6/64	9/8/65	None		
76	6.8**	11/30/67	11/30/67	10.0**	12/6/67	
				8.1 "	12/20/67	
				12.9 "	1/3/68	1 mo.
				13.6 "	1/17/68	
				8.8 "	1/31/68	2 mos.
				8.0 "	2/14/68	
				10.2 "	2/28/68	3 mos.
				12.3 "	3/13/68	
				10.0 "	3/17/68	
				7.5 "	4/10/68	
				13.5 "	4/24/68	5 mos.
				9.6 "	5/8/68	
				8.1 "	5/19/68	
				9.2 "	6/5/68	
				10.7 "	6/19/68	
				6.4 "	7/3/68	7 mos.
				8.0 "	7/17/68	
				12.8 "	7/31/68	8 mos.
				11.4 "	8/14/68	
99	8.4**	11/30/67	11/30/67	17.5 "	12/6/67	
				13.7 "	12/20/67	
				6.4 "	1/3/68	1 mo.
				9.4 "	1/17/68	
				14.4 "	2/14/68	
				12.0 "	2/28/68	3 mos.
				15.4 "	3/27/68	4 mos.
				13.0 "	4/10/68	
				14.2 "	4/24/68	5 mos.
				11.4 "	5/22/68	
				14.4 "	6/19/68	7½ mos
141	None		6/19/68	6.8 "	8/17/68	2 mos.
				15.0 "	8/31/68	
				8.7 "	9/14/68	3 mos.
				10.5 "	9/28/68	
				13.0 "	12/1/68	4 mos.
				8.3 "	12/1/68	

\* I.U./24 hrs.

\*\* mI.U./ml

1001987

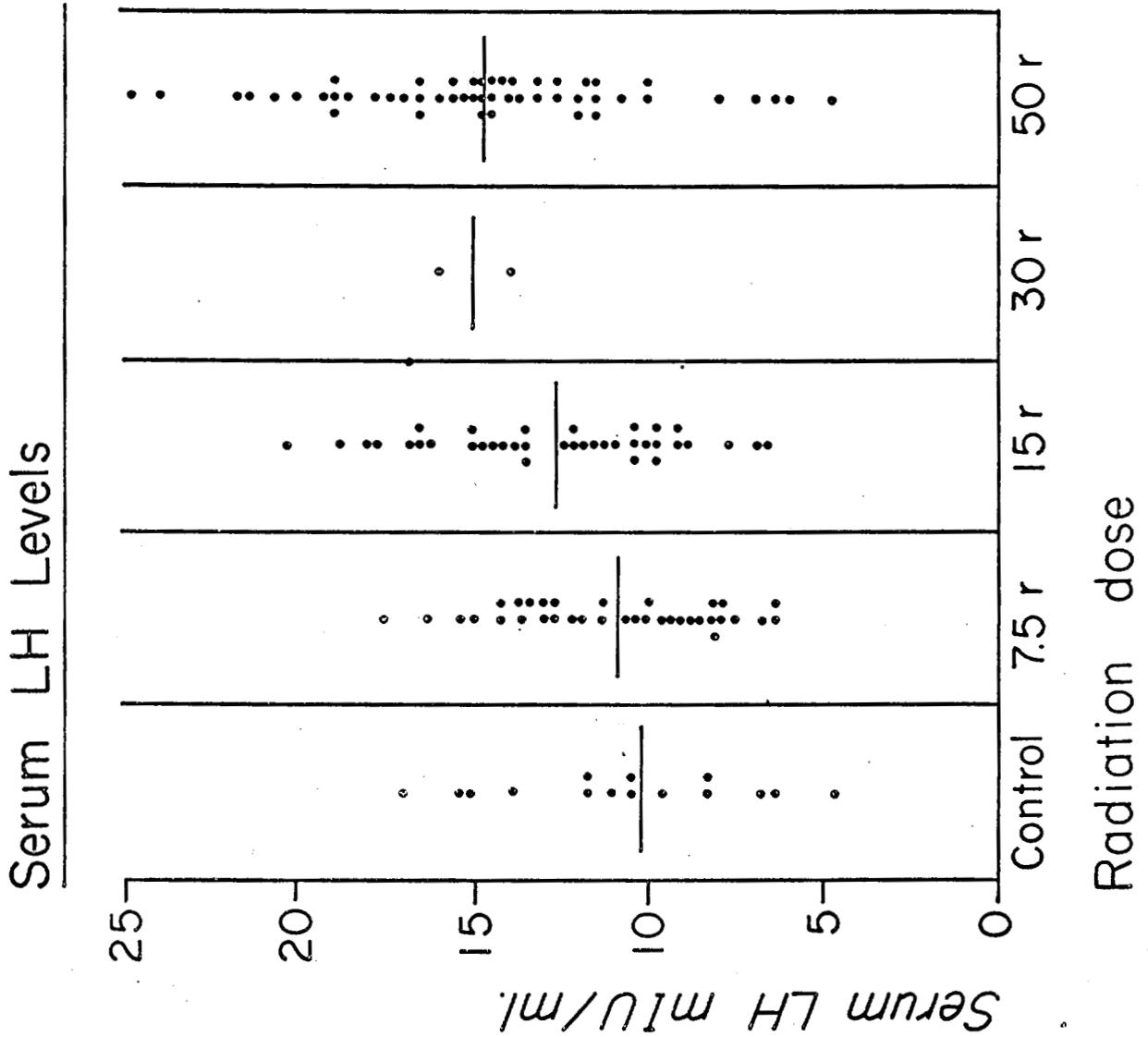
Therefore, the more precise radioimmunoassay method for measuring serum LH levels was established in our laboratory. This technique is based on the method of Morgan as modified for LH determinations by Midgley. The Second International Reference preparation was also used as our standard for this procedure. Our normal range for pre-irradiation serum LH levels was determined from values obtained in 70 normal adult males. The range is 4 to 19 mI.U./ml with a mean of 10.3 m.I.U./ml. Serum specimens for LH measurement were obtained before in most patients and at 2-week intervals following irradiation up to 8 months.

It was not possible to obtain control sera in all instances and post-irradiation sera from the volunteers at the high radiation doses because some of the volunteers began their exposure schedule prior to the availability of LH radioimmunoassay method. Therefore, the fifteen men with control values served as our inmate controls for doses of x-ray ranging from 7.5 to 50 r.

When all the post-irradiation serum LH values are graphically displayed (Fig. 31) for radiation dosage administered it becomes clear that a small but gradual increase in mean LH titer occurs at each increment in x-ray dosage from 7.5 to 50 r. At 15 r the increase in LH levels was noted during the 10th through the 20th week post-irradiation. At 50 r the increase was noted between the 7th and 35th weeks. In three men at this dose level where serial levels were available including control values, the mean increase in LH titers varied from 26 to 230 per cent above basal control levels beginning at the 7th post-irradiation week. Measurements of plasma testosterone from samples obtained during this longer post-irradiation period have not been completed as yet.

In summary then our preliminary study of Leydig-cell function following graded doses of x-ray irradiation to the human testis indicates that prior to seven weeks no changes occur. However, after seven weeks following 50 r a small rise in serum LH titer is detectable suggesting that Leydig cell function has been compromised. The elevation in serum LH levels then represents a compensatory increase presumably to restore testosterone levels to normal.

Figure 31. SERUM LH LEVELS



1001989

VII. Immunologic Studies

This aspect of our project is being studied in collaboration with Dr. William Clarke and his group at Battelle Memorial Institute in Hanford. Animal and human experiments have clearly demonstrated that the germinal epithelium is antigenic. Previous data from our laboratory have shown that sperm counts decrease following a testicular biopsy. This decrease is temporary in nature and affects approximately 40 per cent of individuals subjected to a unilateral testicular biopsy. We proposed that the alteration in spermatogenesis was caused by an induced antigen-antibody reaction. In order to test this hypothesis further additional experiments were designed to see whether or not antibodies could be detected following x-ray irradiation and unilateral testicular biopsy.

Techniques for (1) producing soluble antigen from human sperm, (2) producing rabbit anti-human-sperm antibodies and quantitating serum sperm antibodies using the Ouchterlony plates and fluorescent stained antibody methods.

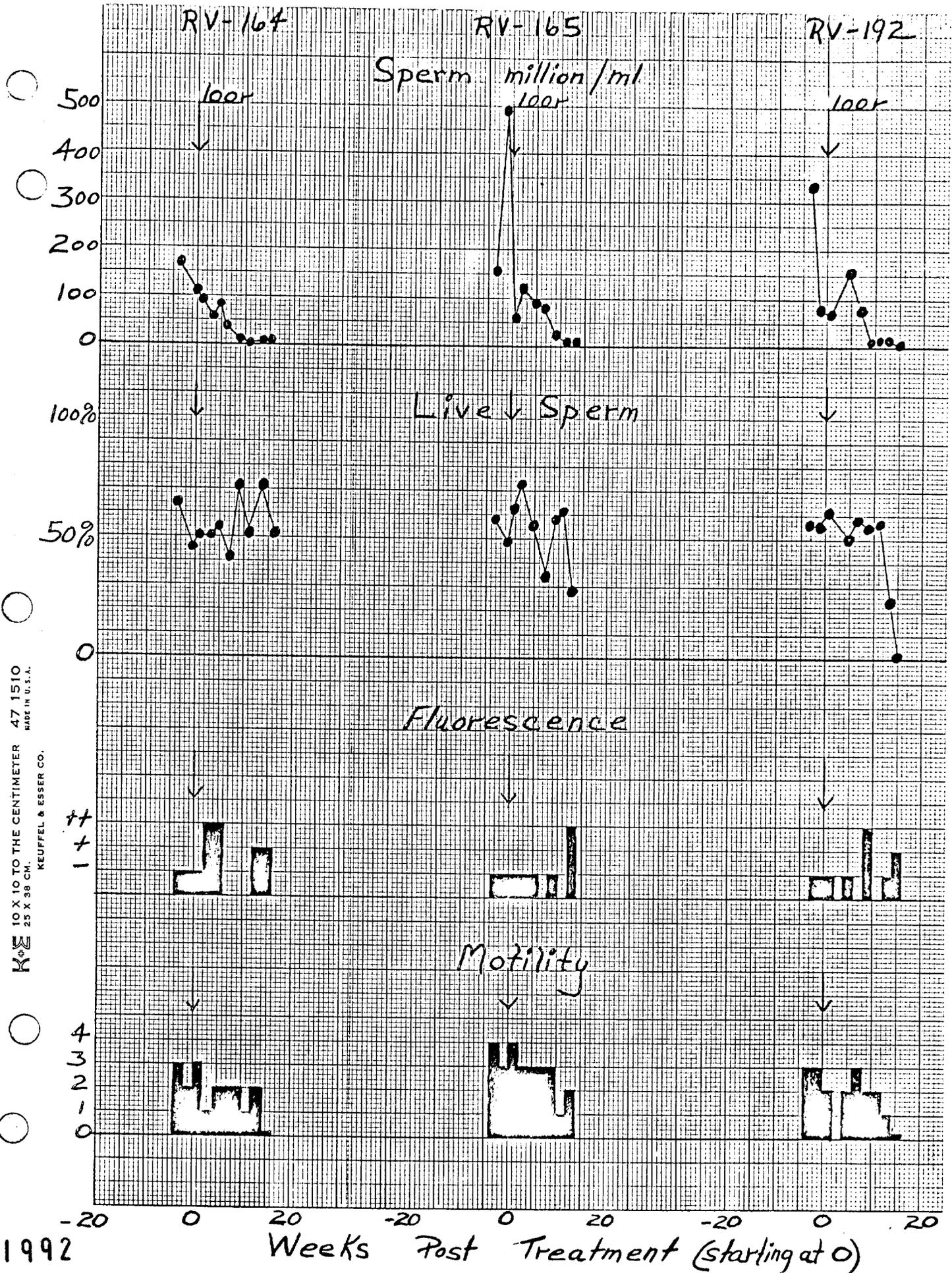
The fluorescent stained antibody method which we used is described in the following sentences. Sperm from non-treated or control subjects is washed three times with 0.5 per cent phosphate buffer solution and suspended in the buffered solution at a concentration of 20 million sperm per ml. Two drops of the sperm suspension is then incubated with two drops of a treated subject's serum at 37° for 45 minutes. Following this the sperm are washed three times with phosphate buffered solution and two drops of rabbit anti-human globulin antisera are added and incubated as above. The treated sperm are then washed three times with the phosphate buffered solution and two drops of sheep anti-rabbit fluorescein conjugated antisera (diluted 1:5) are added and incubated for 45 minutes at 37°. Following this incubation the sperm are washed three times with phosphate buffer solution and then spun dry. The sperm are resuspended in one drop of a 90 per cent glycerol solution. This final suspension is placed on a glass slide and covered with a cover slip. This preparation is then immediately viewed under the ultraviolet microscope and evaluated for fluorescence. For comparison and control of the procedure a previously determined treated patient's serum known to be positive and an untreated patient's serum are viewed with each "run". The light on the UV microscope is adjusted so that the "positive" serum is uniformly fluorescent and the "negative" serum is without fluorescence. The unknown sera are then graded in the following manner: 0 = no fluorescence; 1 + = equivocal fluorescence as judged by two independent observers;

2 + = definite fluorescence as agreed by two independent observers.

Figures 32 through 34 listed our results to date. In addition to the fluorescent studies, observations were made as to sperm concentration in the fresh seminal fluid as well as motility and per cent viable sperm. The experimental groups were divided into controls, biopsy only and exposure to 100 r. Each man in the latter two groups had never received x-ray nor had been biopsied.

None of the control men's sperm became fluorescent. RV 160 exhibited a decrease in sperm count, bizarre sperm morphology and necropermia for two weeks. The explanation for this temporary alteration in his spermatogenesis is not clear. He remained free of febrile illnesses, etc. Each of the men exposed to 100 r demonstrated a fluorescence reaction when their sera were incubated with normal sperm. One of the four men biopsied also demonstrated a positive fluorescent reaction.

Therefore, our preliminary studies indicate that the performance of a testicular biopsy, as well as exposure to x-ray irradiation, initiates an antigen-antibody reaction. The results with the Ouchterlony plate system have been negative to date. This is probably due to the lesser sensitivity of this system. Efforts are now underway with soluble sperm antigen prepared from "pressed" sperm to quantitate our results in a more precise fashion.

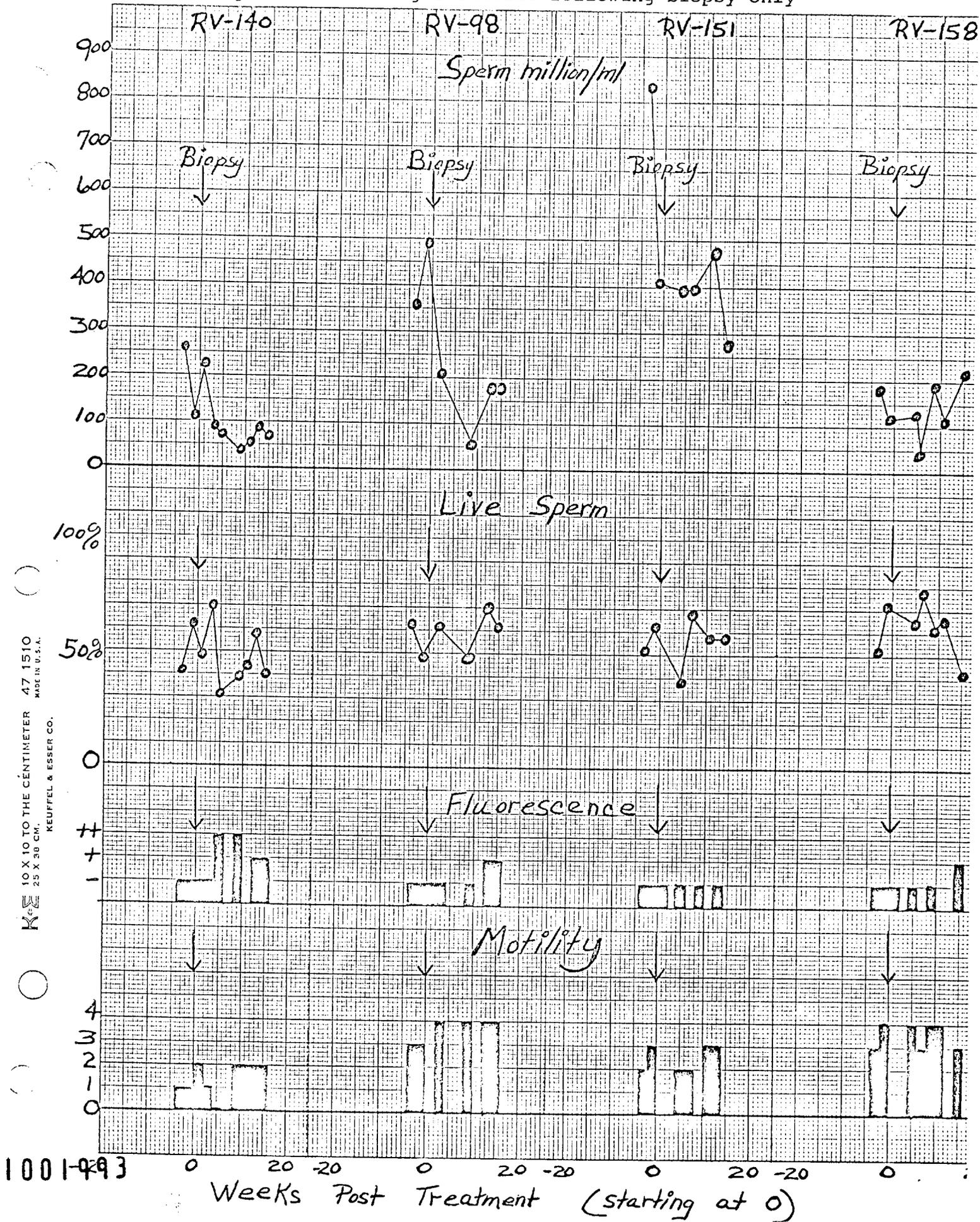


KEUFFEL & ESSER CO.  
 10 X 10 TO THE CENTIMETER 47 1510  
 MADE IN U.S.A.  
 25 X 36 CM.

1001992

Weeks Post Treatment (starting at 0)

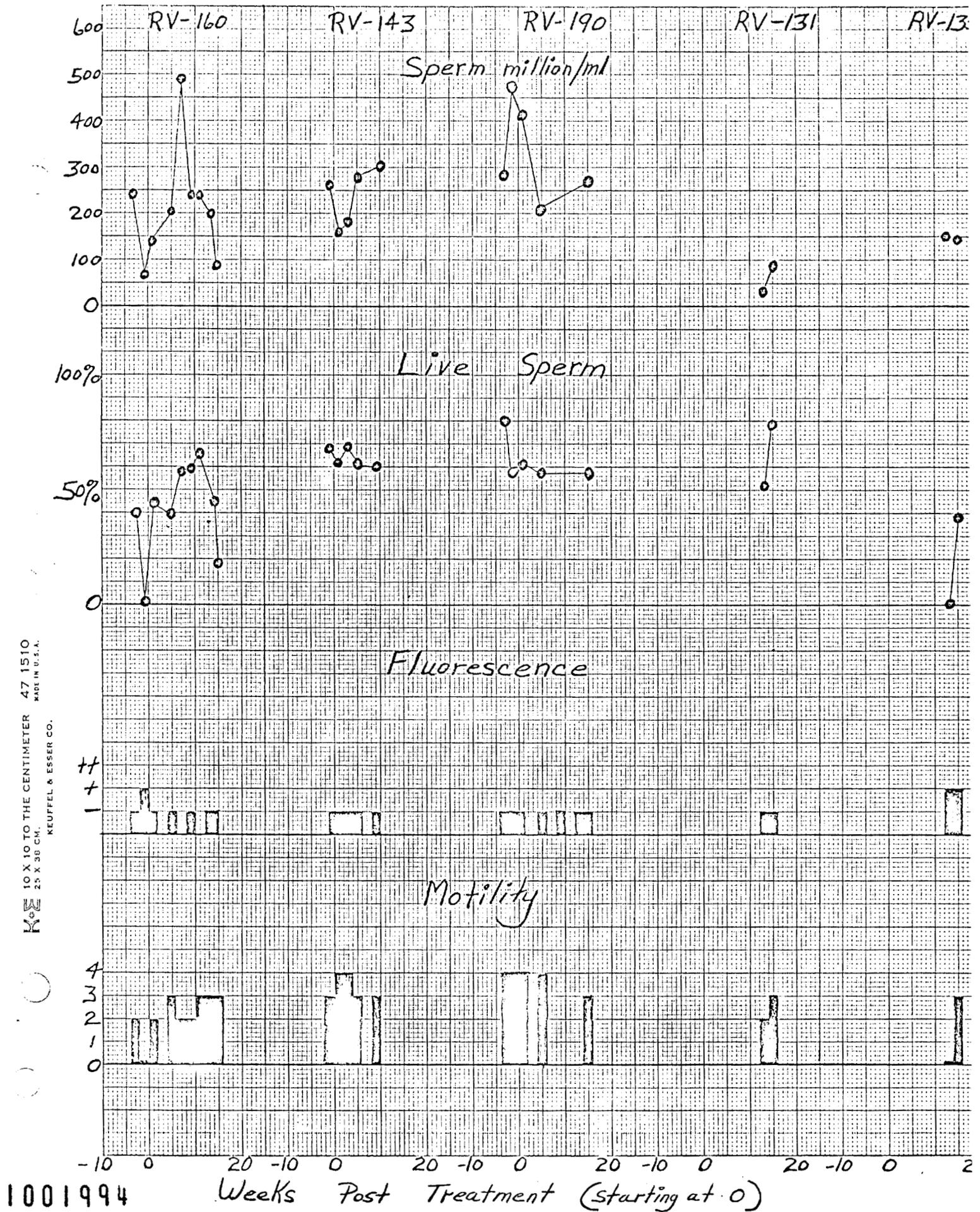
Fig. 33 Immunologic studies following biopsy only



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1001-493

Fig. 34. Immunologic studies following 100 r to the testis



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1001994

VIII. Trainees

The following postdoctoral trainees have worked on various aspects of this program during the period 1963-69. Although their stipends were not directly supported by this contract, the work carried on by the contract contributed heavily to their training. Conversely, they provided valuable professional expertise to the conduct of our work.

Also, Todd Thorslund (Assistant Professor, University of Washington Center for Quantitative Science), who is currently receiving support under this contract, was initially trained in our laboratories and performed much data analysis for this program while a graduate student in the Department of Biostatistics at Johns Hopkins.

Adelina de Souza Matsui, M.D., 1962-64, Associate Professor of Pharmacology, University of Hawaii Medical School, Honolulu, Hawaii

Donald L. Gordon, M.D., 1963-65, Assistant Professor of Medicine, The Chicago Medical School, Mt. Sinai Hospital Medical Center, Chicago, Illinois

Arnold B. Barr, M.D., 1963-65, Associate Chief, Medical Service, USPHS Hospital, Staten Island, New York

George R. Halling, M.D., 1964-66, in private practice (Internal Medicine) Santa Barbara, California

Edward L. Michals, M.D., 1965-67, Assistant Chief of Research, USPHS Hospital, New Orleans, Louisiana

Richard W. Carpenter, M.D., 1966-67, Clinical Instructor of Medicine, University of Kentucky Medical Center, Lexington, Kentucky

Stephen P. Swersie, M.D., 1966-68, Assistant Chief of Medicine (Endocrinology), USPHS Hospital, Boston, Massachusetts.

Frederick Naftolin, M.D. 1966-68, Fellow, Department of Human Anatomy, University of Oxford, Oxford, England.

Duane H. Espeland, M.D., 1967-69, Current Fellow. (effective 7/1/69, Instructor in Medicine, University of Washington School of Medicine, Seattle, Washington)

Richard J. Sherins, M.D., 1967-69, Current Fellow, (effective 7/1/69, Fellow, National Cancer Institute, Bethesda, Maryland)

John M. Leonard, M.D., 1968-70, Current Fellow.

Glenn A. Patchen, M.D. 1968-, Current Fellow.

IX. Degrees Awarded

Kenneth Swinth received his M.S. degree from the University of Washington in 1964, while working on our project. His thesis, "The Design, Shielding and Preliminary Dosimetry for a Neutron Irradiation Facility to Study Spermatogenesis in Man", was included as an addendum to our 1966 comprehensive report.

Arnold Barr received a M.Sc. in 1967 from McGill University working under the mentorship of Yves Clermont. Material for his master's thesis, "Human Spermatogenesis", was derived from specimens obtained for this contract.

X. RLO Series

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 radio-immunoassay 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, C.A. Paulsen. Fertility and Sterility, July 1965.

RLO-1781-3 Effect of X-Irradiation of testis on Plasma Testosterone in Normal Male Volunteers (abstract). 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 (abstract). 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, 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, C.A. Paulsen. American Journal of Obstetrics and Gynecology, January 1967.

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, W.D. Drucker. Recent Progress in Hormone Research, Vol. 24, 1968.

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

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