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Research Project:

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

Request for Renewal

of

AEC Contract #AT(45-1)-225

Task Agreement #6

1970 - 1971

(Terminal Period)

Submitted by

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

Program Director

Authorized Signature
University of Washington

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UNIV. of Washington Agreement #6

Introduction

A total of 64 volunteers have been irradiated with an acute dose of 7.5, 15, 30, 50, 100 or 400 r of x-ray to the testes. Of these, 35 have had both a control testicular biopsy and one or more biopsies at given time intervals following irradiation. Three additional men have had only a post-irradiation biopsy. Twelve more await a final biopsy before release from the study. These will be performed during the anniversary month of each individual's irradiation exposure, as outlined in Table I. To date there have been a total of 200 biopsies, many of these bilateral.

Statistical Analysis

As a general principle for an efficiently conducted scientific experiment, the upper limit of the amount of resources generated to extract information from the obtained data is directly proportional to the amount of resources invested in the data. Using this assumption, it is clear that anything less than a maximal effort in regard to statistical analysis would be inefficient.

It is proposed that each subject who has ever been connected with the study (regardless of the potential modifying factors--such as a testicular biopsy obtained after irradiation--he has been exposed to) be included in the analysis. However, it is suggested that the analysis be carried out in three stages, where each stage has a different set of underlying assumptions. In the first stage the data would be analyzed, making the simplifying assumption that radiation is the only modifying factor. In the second stage we would attempt to adjust for additional modifying factors. Finally, in the last stage all individuals exposed to additional potential modifiers would be eliminated from the analysis.

In the Comprehensive Progress Report of AEC Contract #AT(45-1)-1781, Section II-C, two general parametric models, the "Multivariate One Hit Curve" and the "Multivariate Logistic Curve," were suggested as potential models for the analysis of sperm count data that have been transformed into "all or none" responses.

These models would be utilized at all stages in the analysis and in both the damage and recovery phases. In addition some attempt would also be made to utilize the actual sperm counts. Finally additional parallel estimation of the ED/50/t would be made utilizing non-parametric methods, and the validity of the statistical methods employed throughout investigated by the use of Monte Carlo simulation techniques.

However, it is realized that a number of additional potential methods of analysis are possible. As a result, and due to the uniqueness and value of the data, it is proposed that the data be placed on IBM cards or tape according to various formats and advertised that it is available to interested investigators for the asking.

Germinal Epithelium

From the biopsy specimens obtained, we are continuing to analyze changes in the germinal epithelium during the damage and recovery phase. This has involved quantitation of the changes in the various cell types, as outlined in our
1004472 Comprehensive Progress Report. This time-consuming study is fairly

well up-to-date, and with the addition of the 12 final specimens, outlined in Table I, final conclusions can be drawn based on observations for as long as six years following irradiation.

Other Morphologic Changes

Following the Hiroshima and Nagasaki bombings, acute studies were made of testicular changes in atomic bomb casualties. During succeeding years the Atomic Bomb Casualty Commission has followed the survivors and attempted to correlate estimated dose and distance from the hypocenter with alterations in testicular histology. A report of their findings has been given in Atomic Bomb Casualty Commission Technical Report 15-66, and Archives of Pathology 82:542-54, 1966. In this study they defined six histologic parameters as follows:

1. Generalized atrophy
2. Tubular wall thickening
3. Tubular sclerosis
4. Interstitial fibrosis
5. Interstitial-cell hyperplasia
6. Vascular hyalinization

Of these six categories they found significant differences in the prevalence of tubular sclerosis and vascular hyalinization between Hiroshima survivors within 1400 meters from the hypocenter, who had received 300 rads or more, and comparison groups.

We stated in our April 1969 Comprehensive Progress Report that evidence of vascular change following 400 r was observed. Since then we have noted changes at lower doses. These changes have yet to be quantitated and correlated with radiation dose, duration of time since exposure and the gonadotrophic hormone levels. Our phasing-out studies will concentrate on such analysis.

The protocol as outlined in the ABCC Technical Report 15-66 will be followed both for the tubular sclerosis and the vascular hyalinization. The classification is as follows:

- | | |
|-------------------------|--|
| Tubular Sclerosis: | 0 Less than 3% of tubules sclerotic |
| | 1 3%-10% of tubules sclerotic |
| | 2 10%-25% of tubules sclerotic |
| | 3 25%-50% of tubules sclerotic |
| | 4 50% or more of tubules sclerotic |
| Vascular Hyalinization: | 0 No arteries identified to show medial hyalinization |
| | 1 Rare small arteries show medial hyalinization |
| | 2 A few small arteries show medial hyalinization |
| | 3 Moderate numbers of small arteries show medial hyalinization |
| | 4 Many small arteries show medial hyalinization |

Since we know precisely the rad-dose to the testis, and since we can correlate changes with each individual's control testicular histology, we will be able to establish a Time-Dose-Response relationship for hyalinization, or premature "aging," in both the seminiferous tubular wall and the testicular vascular system, if such a relationship does exist.

In addition, we now have the facilities of an electron microscope through the Department of Biological Structure of the University of Washington and microtome facilities at the USPHS Hospital. Dr. David de Kretser, one of our postdoctoral research fellows, is well-trained in the use of the electron microscope as well as in the fine structure of the testis. He is anxious to obtain material from the remaining post-irradiation biopsy specimens. This may well provide additional information regarding subtle basement membrane or vascular changes, particularly during the longer-term follow-up studies.

Urinary Follicle-Stimulating Hormone (FSH) Studies and Seminal Fluid Analysis

Three of the volunteers still have elevated urinary FSH titers, and two have equivocal or borderline values, in that they have not yet stabilized. These will be followed at monthly intervals until termination date, as outlined in Table II. Those who have definitely stabilized will have one or two further determinations, the final one being immediately prior to biopsy and termination.

The schedule outlined in Table II calls for 32 48-hour urine collections beyond April, 1970. This will require a minimum of 225 rats (\$250) for analysis. This schedule will permit us, as well as possible, to correlate urinary FSH excretion with sperm production and testicular histology.

We will continue to collect weekly seminal fluid specimens until date of final biopsy.

Project Personnel Not Supported by This Contract

During the years of our AEC contract we have had from 2 to 5 postdoctoral research fellows working on various aspects of the radiation program. Each of these fellows has contributed from 15-25% of his time directly to the program, although no salaries or stipends have been paid from contract funds. In the early years of the project, Dr. Gordon as a fellow devoted up to 75% of his total time to this program. During the year to come, it is estimated that Dr. David de Kretser, Dr. Richard Santen and Dr. Duane Espeland will each devote 10% of their time to this research--Dr. de Kretser in histology and electron microscopy studies as mentioned above, Dr. Santen in assay procedures and problems and Dr. Espeland in various areas of the program. No funds are requested for support of these investigators; this represents a contribution equivalent to more than \$4000 in professional salaries. Professional time contributions in previous years have ranged up to \$6500 or more per year in dollar amounts.(does not include Dr. Paulsen).

Other personnel working in our laboratory who will perform services without AEC financial support are:

Margaret Couture	}	Technician, histology lab	25% time	(\$1742)
Ofelia Francisco		Laboratory assistant	25% time	(\$1177)

In addition, other personnel will contribute effort from time to time as their services are required.

TABLE I

PROPOSED BIOPSY AND TERMINATION SCHEDULE

RV #	X-Ray Date	Dose	Planned Biopsy Date	Time Interval
26	6/24/64	400 r	June 1970	6 years
4	9/9/64	100 r	September 1970	6 "
32	5/3/65	"	May 1970	5 "
74	9/22/65	"	September 1970	5 "
149	9/11/68	"	September 1970	2 "
192	12/11/68	"	December 1970	2 "
78	11/30/67	50 r	November 1970	3 "
147	6/19/68	"	June 1970	2 "
144	6/19/68	"	June 1970	2 "
133	6/19/68	"	June 1970	2 "
31	2/19/65	15 r	February 1971	6 "
141	6/19/68	7.5 r	June 1970	2 "

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TABLE II.

ANTICIPATED URINE COLLECTION AND BIOPSY DATES FOR FSH FOLLOW-UP STUDIES

RV #	Termination Date	Current FSH Status	Planned Urine Collection Dates												
			Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb		
26	June	Normal			UBV	U	U	UBV							
4	September	Borderline					U	UBV							
32	May	Normal	U	UBV			U								
74	September	Normal				U	U	UBV							
149	September	Elevated	U	U	U	U	U	UBV							
192	December	Elevated	U	U	U	U	U	U							
78	November	Normal				U	U								
147	June	Equivocal	U	U	UBV										
144	June	Elevated	U	U	UBV										
133	June	Normal		U	UBV										
31	February '71	Normal												U	
141	June	Normal		U	UBV										UBV

U = 48 hr Urine Specimen
 B = Biopsy
 V = Vasectomy

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