

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)

DOE-RICHLAND
GSS HUMAN TEST SUBJECTS STUDIES
PRISONER STUDY

046264

1780 - HELLER PACIFIC NW RES. FOUNDA.

ASSIGNED NUMBER: RLHTS 94-0059

REPOSITORY:
COLLECTION:

BOX:

FOLDER:

3003024

Submitted by

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

C. Alvin Paulsen

Program Director

George W. Farwell

Authorized Signature
University of Washington

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 April 1969 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
Ofelia Francisco	Laboratory assistant	25% time

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

TABLE I

PROPOSED BIOPSY AND TERMINATION SCHEDULE

<u>RV #</u>	<u>X-Ray Date</u>	<u>Dose</u>	<u>Planned Biopsy Date</u>	<u>Time Interval</u>
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									
4	September	Borderline					U	UBV								
32	May	Normal	U	UBV			U									
74	September	Normal					U	UBV								
149	September	Elevated	U	U	U	U	U	UBV								
192	December	Elevated	U	U	U	U	U	U	U	U	UBV					
78	November	Normal				U						UBV				
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

Terminal Budget
Contract AT(45-1)-2225, Tast Agreement 6
May 1, 1970-April 30, 1971

	<u>AEC</u>	<u>U of W</u>
SALARIES		
a) <u>Professional</u>		
C. Alvin Paulsen, M.D., Associate Professor, 15% time		
Todd Thorslund, Sc.D., Biostatistician, 25% time		
b) <u>Non-professional</u>		
Research Technologist II, Donald Moore, full-time		
Research Technologist I, Constance Lowe, full-time		
Statistical Assistant, Marian Ursic, 87 1/2% time		
Secretary IV, Geneva Green, 25% time		
Total salaries		
SALARY COSTS: State retirement, TIAA premiums, FICA, Medical Aid and Life/Health Insurance premiums		
SUPPLIES		
Immature female rats for bioassay		
TRAVEL		
One staff member, six trips to Walla Walla		
VOLUNTEER STIPENDS		
a) 12 inmate volunteers @\$10/mo until off program		
b) 12 vasectomies @\$100/inmate and \$60/physician		
c) 12 biopsies @\$25		
Total stipends		
MISCELLANEOUS SERVICES		
a) Shipping specimens		
b) Computer time		
Total miscellaneous		
INDIRECT COSTS: 27% of salaries and wages		
TOTAL BUDGET	43,318	5,416

CURRICULUM VITAE:

NAME: DR. David Morritz de Kretser.

DEPARTMENTAL ADDRESS:

PRIVATE ADDRESS:

Department of Anatomy,
Monash University,
Clayton, Victoria 3168
Australia.

DATE OF BIRTH: 1939 at Colombo, Ceylon.

NATIONALITY: Australian.

MARITAL STATUS: Married. 2 children aged 5 years, 2 years.

ACADEMIC RECORD:

A. MEDICAL COURSE UNIVERSITY OF MELBOURNE (1957-1962)

Honours during course:

- 1st. class in Physics and Chemistry (1st year)
- 2nd. class in Biochemistry & Physiology (2nd.yr)
- 1st. class in Biochemistry (3rd. year)
- 2nd. class in Physiology including Exhibition in Physiology (3rd. year).
- 2nd. class in Pathology, 3rd. class in Microbiology (4th. year).

Final Examination:

- 1st. class in Surgery, including Exhibition.
- 3rd. class in both Medicine, Obstetrics and Gynaecology.

Position in class of 180 at Final Examination: 2nd.

GRADUATED: Bachelor of Medicine, Bachelor of Surgery
(M.B., B.S.) 1962.

B. POSITIONS SINCE GRADUATION:

1963: Junior Resident Medical Officer, Prince Henry's Hospital, Melbourne, with terms in Medicine, Surgery, Casualty and Anaesthetics.

- 1964: Senior Resident Medical Officer, Prince Henry's Hospital with terms as Hospital Admitting Officer, Orthopaedic Surgery, Gynaecology and Ear, Nose and Throat Diseases.
- 1965: Demonstrator Department of Anatomy, Monash University.
- 1966-1968: Lecturer, Department of Anatomy, Monash University.
Clinical Assistant, Endocrine Clinic, Royal Women's Hospital, Melbourne.
- 1968: Senior Lecturer, Department of Anatomy, Monash University.

C. ACADEMIC RECORD SINCE GRADUATION:

- 1966: Passed Primary Examination for Royal Australasian College of Surgeons.
- 1967: Holder of National Health and Medical Research Council Grant for investigation into the Structure and function of the human testes and its response to gonadotrophic treatment.
- 1968: Thesis for Doctor of Medicine ready for submission.
- 1968: Recipient of grant from Rural Credits Fund of Australia.

D. PUBLICATIONS:

1. The fine structure of the testicular interstitial cells in men of normal androgenic status. Z. Zellforsch., 80: 594, 1967.
2. Endocrine and histological studies during human pituitary and chorionic gonadotrophin treatment of oligospermia. With H.P. Taft. Med. J. Aust., Vol. II for 1967, p. 513. Proc. Aust. Endocrine Society, 1967.
3. Changes in the fine structure of the interstitial cells of the human testis after treatment with human pituitary and chorionic gonadotrophin. Z. Zellforsch., 83, 344, 1967.
4. Endocrine and histological studies on oligospermic men treated with human pituitary and chorionic gonadotrophin. With H.P. Taft, J.B. Brown, J.H. Evans, and B. Hudson. J. Endocr.: 40; 107, 1968.
5. The fine structure of the human testis in hypogonadotrophic hypogonadism. Proc. Aust. Conf. on Electron Microscopy, p. 53, 1968.

6. Ultrastructural studies on the human Sertoli cell. J. Anat. (Lond.), 103: (In Press), 1968. Proc. Anatomical Society of Aust. and New Zealand. 1967.
7. Ultrastructural features of human testicular interstitial cells. J. Anat. (Lond.), 103: (In Press), 1968. Proc. Anatomical Society of Aust and New Zealand. 1967.
8. Crystals of Reinke in the nuclei of human testicular interstitial cells. Experientia 24: 587, 1968.
9. The clinical uses of human gonadotrophins. With H.P. Taft, F.D. Adey, J.B. Brown, J.H. Evans, and B. Hudson. Aust. Ann. Med. (In Press), 1968.
10. The fine structure of the immature human testis in hypogonadotrophic hypogonadism. Virchow's Archives (In Press), 1968.
11. Studies on the autoradiographic localisation of I¹²⁵ labelled luteinizing and growth hormone in the immature rat. With H.G. Burger, K.J. Catt, and G.C. Smith. J. Anat. (In Press), Proc. Anatomical Society of Australia and New Zealand (1968).
12. The human testicular interstitial cell. Demonstration at 2nd. Conf. of New Zealand Society for Electron Microscopy, Dunedin, 1967.
13. Autoradiographic Studies on the localisation of I¹²⁵ labelled human luteinizing and human growth hormone in immature male rats. With K.J. Catt, H.G. Burger, and G.C. Smith. Submitted to J. Endocrinol.
14. The conversion of cholesterol to Androgens by Rat Testes; Comparison of interstitial cells and seminiferous tubules. With D. Irby and P.F. Hall. Submitted to Endocrinology.
15. Histochemical demonstration of Δ^5 3β Hydroxy-steroid dehydrogenase activity in the developing rat testis, with D.W. Lording. Proc. of Australian Endocrine Society in Med. J. Australia, In Press.

16. On the presence of immunoreactive growth hormone in a bronchogenic carcinoma. With D.P. Cameron, H.G. Burger, K. J. Catt and J.B. Best. Aust. Ann. Med. (In Press).
17. Ultrastructural features of human spermiogenesis. Z. Zellforsch. (In Press).
18. Ultrastructural features of human spermiogenesis. Proc. of Anatomical Society of Aust. and New Zealand. J. Anat. (Lond.) Abst. (In Press).
19. Interstitial cells in the developing rat testis. With D.W. Lording. Proc. of Anatomical Society of Aust. and New Zealand. J. Anat. (Lond.) Abst. (In Press).
20. Thesis: "Studies on the structure and function of the human testis". Submitted for Doctorate in Medicine at Monash University, Clayton, Victoria, Australia.

CURRENT APPOINTMENT: Senior Fellow in Medicine, University of Washington School of Medicine, effective 9/18/69. (NIH International Postdoctoral Fellowship awardee.)

DON SAY IT --- Write It!*UW*DATE April 1, 1970TO Dr. E. L. AlpenFROM C. L. RobinsonORIGINAL SIGNED BY
C. L. ROBINSON

Ed - DBM, confirming Goldstein's call to you, has advised RL of plans to terminate Contract AT(45-1)-1781 with Paulsen, University of Washington.

UW holds title to the Walla Walla equipment; however, there is good indication of willingness to transfer custody to BNW. If you will let me know when you are ready, Mrs. Nell Fraser, RL, will handle equipment transfer arrangements. (I am reasonably certain that UW won't care to absorb moving costs, so please plan this charge in your budget.)

cc: N. W. Fraser
C. R. Qualheim
M. W. Tiernan
L. C. Brazley

APR 2 - 1970

+ "SOME LEARN FROM EXPERIENCE, SOME NEVER RECOVER FROM IT" +

3003035

March 31, 1970

Joseph D. Goldstein
Division of Biology & Medicine, HQ

CONTRACT NO. AT(45-1)-2225, TASK AGREEMENT NO. 6,
UNIVERSITY OF WASHINGTON

Enclosed for your consideration is a proposal for extension of the subject project entitled "The Study of Irradiation Effects on the Human Testis Including Histologic, Chromosomal and Hormonal Aspects." Dr. C. Alvin Paulsen requests an AEC support level of \$81,278 for the program during the period May 1, 1970 through April 30, 1971.

As you are aware, the University of Washington has determined that the human neutron studies which were originally proposed will not be conducted. Following my telephone conversation with J. C. Whitnah on March 30, I discussed with the University of Washington administration the possibility of transferring the neutron generator from Washington State Penitentiary to Pacific Northwest Laboratory for studies on spermatogenesis in animals. It appears that we will be able to arrange for transfer of the equipment.

Nell W. Fraser
Assistant Contract Administrator
Contracts and Supply Division

Enclosures:

1. Research Proposal (4)
2. Let, 3/20/70
Farwell/USAEC

cc: C.L. Robinson
L&U Division, RL

Distribution:

- 1-Addressee
- 2-Robinson
- 3-CA OFF
- 4-CA RDG
- 5-C&S RDG

AIRMAIL

C&S:GA
Fraser/RL
3/31/70

3003056

DONT SAY IT --- Write

OFFICIAL USE ONLYDATE March 30, 1970TO Nell FraserFROM C. L. Robinson

According to my understanding of Goldstein's (DBM) 3/27/70 telephone call to Alpen (BNW), DBM plans to discontinue AT(45-1)-1781 with Paulsen (UW). I further understand that DBM plans discussion with UW concerning possible UW agreement to transfer selected related equipment to BNW.

This DSI is intended only as information.



cc: S. H. Tumlinson
H. E. Parker
C. R. Qualheim
L. C. Brazley/M. W. Tiernan

OFFICIAL USE ONLY

"SOME LEARN FROM EXPERIENCE, SOME NEVER RECOVER FROM IT"

3003037

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98105

March 20, 1970

Office of the Vice President for Research
203 Administration Building

AIR MAIL

U. S. Atomic Energy Commission
Washington, D. C. 20545

Gentlemen:

Enclosed is a proposal from Associate Professor C. Alvin Paulsen, Department of Medicine, School of Medicine, requesting the renewal of Contract No. AT(45-1)-2225, "The Study of Irradiation Effects on the Human Testis Including Histologic, Chromosomal and Hormonal Aspects."

Although the proposed extension of Professor Paulsen's earlier work on the effects of X-ray irradiation on spermatogenesis in man has been approved by the University's Biomedical Sciences Review Committee, I should inform you that continuation of the program using inmates of the Washington State Penitentiary as subjects will require the approval of the Department of Institutions of the State of Washington. Such approval has been requested and is now under consideration by the Department.

Budgetary provisions have been reviewed and approved by Mr. Donald R. Baldwin, Director, Office of Grant and Contract Services. The sum of \$81,278 is requested for the period from May 1, 1970, through April 30, 1971.

It is a pleasure to approve this proposal for the University of Washington and to transmit it to you for your consideration.

Sincerely yours,



George W. Farwell
Vice President for Research

GWF:rs
Enclosure

cc: Dean Robert L. Van Citters, School of Medicine
Professor Robert G. Petersdorf, Chairman, Department of Medicine
Associate Professor C. Alvin Paulsen
Mr. Donald R. Baldwin
Mrs. Nell W. Fraser, AEC, Richland Operations Office

3003038

MAR 23 1970