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Research Project:

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

Submitted by

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## TITLE OF PROJECT

Effect of Irradiation on Human Testis: Changes in Spermatogenesis, Chromosomal Morphology, Leydig-cell Function and Pituitary-Gonadal Interrelationships as a Function of Radiation Dose.

## II. INSTITUTION SPONSORING INVESTIGATION

University of Washington School of Medicine, Department of Medicine.

## III. SCIENTIFIC BACKGROUND AND MOTIVATION FOR INITIATION OF STUDY

My interest in this problem dates back to April, 1962. At this time I was asked to evaluate the gonadal consequences in three men who were exposed to neutron radiation at the Hanford Atomic Energy Plant. It became apparent, after reviewing the literature, that our knowledge concerning the effects of irradiation, particularly neutron, on the human testis was virtually non-existent. Indeed, all observations had been made "after-the-fact" when industrial exposure had occurred, or following the Hiroshima and Nagasaki episodes (1)(2)(3)(4)(5)(6). Since both dosimetry and the pre-exposure status of the gonads involved were either uncertain or unknown, these data are incomplete from a predicted scientific point of view. As my group and I delved into this problem, the obvious importance of having complete fundamental data on this problem was brought into sharp focus. Aside from the evident hazards of atomic weaponry, the problems of industrial exposure and space travel require attention. Our preliminary studies on the Hanford men were presented at the recent CSU meeting, International Symposium on the Effects of Ionizing Radiation on the Reproductive System. This summary is appended to this application as enclosure (1).

The basic scientific data obtained from this project will have a very important practical value. In our atomic age society, there is always present the possibility of radiation accidents resulting in significant radiation dose to one or more people. The ultimate accident would be a nuclear war involving multitudes of people. Medical treatment of such people and the analysis, for legal reasons, of possible damage to these people, must be based on factual knowledge of the effects of radiation. It is particularly necessary that one know the relationship between the absorbed doses, a physical quantity describing the interaction of the radiation with matter, and the resulting biological effects. In accidents one can estimate the absorbed dose from radiation meters worn by the persons or from measurements made at the scene of the accident. These form our main basis for estimating the possible damage and may be of considerable help in determining the course of medical treatment.

Several questions have arisen concerning this proposed research project. Why use human males as research subjects? And why use neutron irradiation as our main source? First of all, our aim, as is the case of all clinical investigation, is to obtain accurate data that will enable the medical profession to understand "spontaneous" or induced disease processes in the human being. And as a logical consequence, to devise means by which one can alleviate such disease processes. While it is certainly true that there is considerable data, particularly concerning the effects of x-rays, on the effects of irradiation in animals (7)(8)(9)(10). This data cannot directly

relate this to the human male with security. Indeed, basic differences have been suggested between the response of the mouse and rat. For example, Oakberg attributes spermatogonial cell death as the main reason for depletion of the germinal epithelium in the mouse after exposure to x-rays. On the other hand, Jones considers inhibition of mitotic activity as the main cause in the rat. Furthermore, the rate of spermatogenesis is different in man as compared to various animals (and the rate is also different between various lower animal species). This undoubtedly will be reflected in differences in response other than just time-relationship; at least there is no definite data concerning this point in man.

Secondly, neutrons have been selected as our primary object of study for several reasons other than the aforementioned practical aspects. Accurate calculations as to the RBE of neutron irradiation in human beings, especially with respect to gonadal tissue, are not possible due to inadequate data. It would seem highly important from all viewpoints to correct this situation. And, as proposed, our project should supply important data on this point.

Another important reason for studying neutron irradiation is that there are certain difficulties at present in the measurement of neutron dosimetry that need clarification. Dr. Roesch's approach to this problem will provide important data on this point.

Investigators have been cautious, and rightly so, concerning the use of neutrons for human study. Certainly, Stone's work emphasizing the problem of skin ulcerations is an important complication to consider. However, Bewley et al (13) have recently published the results of their experiments dealing with effects of pig skin after neutron irradiation. They point out that the main problem was inadequate understanding of chronic dose fractionation. This would not be a problem with our proposed exposure to a single acute dose. Also, their acute data will serve as a useful guideline in our work. Another problem to consider for the proper use of neutrons is adequate shielding of the subject. Our plan with respect to solving this will be outlined later on in this proposal.

In addition to the neutron aspect of our study, we plan to investigate the effects of radium irradiation on the testes. This source was selected since the dosimetry is well-known and reproducible (14)(14a). Also there are the classic animal experiments of Regaud (15) for guidance as to dose and response and for comparison with our human data.

As an alternative to the use of radium in our preliminary work, x-rays could be used as a source of irradiation. Human data is also incomplete in this area (16)(17)(18), and as mentioned before, there is considerable animal data available for comparison.

~~We would prefer to use radium for our initial work because of the ease of handling and the equipment involved.~~

An important aspect of our study is that concerning chromosomal damage as a result of irradiation. Gradually the literature is developing on this point (19)(20)(21)(22). In addition to the documentation of abnormal chromosomal morphology with reference to dose, extent of damage and subsequent repair, there is need to study cell death as it relates to

chromosomal damage. Sacher<sup>(23)</sup>, among others, has suggested that the well-known effect of irradiation on the aging process is related to chromosomal "breaks."

We have perfected our tissue culture techniques with respect to testicular tissue so that this aspect can be studied. In our investigation of the men at Hanford we have observed chromosomal deletions in F. L., who was exposed to a gonadal dose of 120 rem. This was observed in tissue obtained by biopsy eight months after exposure to mixed neutron and gamma irradiation.

Finally, there is the problem of what constitutes an adequate number of subjects for adequate documentation of the response to any given dose of irradiation. This question is difficult to answer in a dogmatic manner. In my experience, the previously normal human testis responds in an amazingly uniform fashion to agents which alter its function. Therefore, I would consider 6 - 8 subjects in any given category as being an adequate number for statistical reference.

#### IV. SCIENTIFIC SCOPE OF PROPOSED RESEARCH

A. Principal Aims: 1) To relate radiation dosage to changes in gonadal function. 2) To utilize the radiation gonadal changes as means for studying anterior-pituitary interrelationships. 3) To explore therapeutic and medical protective measures with respect to gonadal irradiation. 4) To explore our current concepts concerning radiation dosage expressed in physical terms and their relation to biologic effects.

#### B. Method of Procedure:

1. Research Subjects: Healthy male volunteers who are inmates at the Washington State Penitentiary in Walla Walla will constitute our research material. Approval for this project has been obtained from Mr. Rhay, Superintendent of the State Penitentiary; Dr. Heyns, Director of State Institutions; Dr. Hogness, Assistant Dean of the Medical School; Dr. Kirby, Chairman of the Clinical Research Committee at the Medical School. Copies of their letters are appended as enclosures (2), (3), (4) and (5). In addition, Dr. Bucove, Head of the State Department of Health, has been apprised of this proposed project and he has stated that there are no objections as far as he is concerned.

The aims of our project and its implication with respect to future fertility will be discussed in detail with each inmate volunteer and so documented. Those inmates who agree to become research subjects for our project will be compensated only in the following manner: 1) A stipend of five dollars per month while a research subject and available for observations, 2) a stipend of twenty-five dollars that month in which a testicular biopsy is performed.

The age range contemplated for our research subjects will be from 21 to 45 years. Only those inmates who will be imprisoned at least five years will be selected for our project. After preliminary screening, Dr. Heffern, the prison physician, has informed me that there will be sufficient inmates available. We plan to have 40 to 60 inmates in the program at

may be divided into three phases. Phase I will consist of using radium needles applied to the gonad externally in the same manner (i.e. plastic mold) as for radiation therapy. The dosage range we plan to cover will be from 80 to 800 r. This phase of our study will serve to standardize our radiation techniques and compare our human data with available animal data. As soon as we have developed proper shielding, we will begin to use our neutron source (Phase III).

Phase II will be concerned both with proper shielding and pertinent dosimetry.

Since the University does not have a department of Radiation Physics, the assistance of Dr. W. C. Roesch of the Radiological Physics organization at Hanford has been obtained for the physics of our project. Since we wish to expose only the gonad to significant neutron irradiation, it will be necessary to devise proper shielding for the research subject's body. For this purpose the neutron generator will first be installed at the Hanford laboratory.

We propose to purchase a neutron generator of the type now being used for neutron activation analysis. This will provide the necessary high output. Most of the work will be done with 14 MEV neutrons. Some of the work will be conducted with 2.5 MEV neutrons but at a lower output for comparison effects. The necessary shielding and calibration of the system will be performed at Hanford using mannikins. Dr. Roesch's approach to the shielding problem is as follows: First of all, the machine and the subject being irradiated will be surrounded by enough water, in tanks, to reduce the external radiation to safe levels for the operators and other people in the building. This, then, can be considered a large mass of water surrounding the neutron source. Inside this mass will be a small empty tube enclosing the neutron source at one end and the testes being irradiated at the other end. Thus the rest of the subject's body will be protected by water. As a necessary refinement to this basic "shield," various elements with high cross sections for neutron absorption for 14 MEV neutrons will be studied for use as additional shielding. Also, alterations in the deuteron beam will be studied to permit bringing the testis closer to the source. Then the completed system will be set up at the penitentiary in a basement room of the hospital building, which has been set aside for our use.

Phase III will involve testicular irradiation using our neutron source. The anticipated gonadal dose will range from 10 to 100 rads delivered acutely.

For both Phase I and III of our project, the approach to studying the effects of radiation on human testicular function will be directed along the following general outline. Control observations will be made on each volunteer inmate as to his testicular function. The parameters to be measured will include urinary gonadotrophin and estrogen excretion titers, repeated seminal fluid examinations and microscopic examination of a testicular biopsy specimen. In addition, some of each testicular specimen will be submitted for tissue culture and subsequent evaluation of the chromosomal pattern and morphology. After the acute exposure to radiation, repeat observations of each parameter will be made at appropriate intervals. Thus it should be possible to relate radiation dose to changes in testicular function. More specifically, our aim is to determine the dose by which spermatogenesis is significantly inhibited and to approximate the dose that permits full

recovery. Concerning the latter point it will be necessary to observe each inmate who receives the higher dosages for at least two years; therefore, care will be taken in inmate selection so that this will be possible.

Repeat tissue culture and subsequent chromosomal study will be made after irradiation to evaluate fragmentation, "breaks" and changes in chromosomal numbers. These alterations have been demonstrated to occur after exposure to radiation.

It is generally considered that short of causing cell death, Leydig-cell function remains intact after radiation<sup>(24)</sup>. Urinary estrogen excretion will be studied throughout our project as a reflection of Leydig-cell function. Furthermore, newer techniques for measuring testosterone excretion in the urine will be used to strengthen our study<sup>(25)</sup>.

If it is true that Leydig-cell function remains intact, then we will have a unique opportunity to study urinary gonadotrophin excretion from a qualitative viewpoint in men having selective alteration in germinal epithelial activity. Assays will be made as to total gonadotrophin and luteinizing hormone titer changes. The latter should remain at titers compatible with normal men, whereas the total gonadotrophin levels should rise when significant depression of spermatogenesis occurs<sup>(26)</sup>.

This is, in fact, what has been observed in the men we have followed at Hanford. By enlarging our data we shall be able to firm up this point. Or if this isn't confirmed, determine the reason why not.

After we establish with reasonable degree of certainty as to the relation between radiation dose and testicular changes, it will be feasible to explore possible means for protecting against even temporary testicular damage. For example, it may be possible to protect the germinal epithelium from damage to at least modest irradiation doses by suppressing spermatogenesis. An easy approach would be to inhibit gonadotrophin secretion by the administration of testosterone. This approach was investigated in a preliminary fashion by Joel<sup>(27)</sup>.

With respect to our final aim, it should be possible to study in detail our histologic material (testicular biopsy specimens) in light of the radiation dosage and relate this quantitatively to the number of seminiferous tubules involved. For example, in the testicular material from one of the men at Hanford, some of the seminiferous tubules are populated only by Sertoli cells after exposure to irradiation, whereas other tubules are damaged to a much lesser degree. By applying the principles suggested by Rossi<sup>(28)</sup>, the current concepts regarding the effects of local energy density could be evaluated.

If this application receives favorable action and the support monies become available by July or August of this year (1963), Phase I and II should be well underway by the fall, and it is estimated by Dr. Roesch that by June 1964, Phase II should be completed. Then the neutron apparatus would be transferred to Walla Walla for Phase III studies.

## VI. OTHER PERSONNEL

There are three AEC Radiological Physics Fellows at the University of Washington who have had a second year of their fellowship approved. They have agreed to work on Phase II of this project at Hanford as a means of fulfilling the thesis requirements for their Masters degrees. It is estimated that they will spend full-time on this project for at least a period of six months.

The research technician specified in this application will have the following duties: 1) Assist in supervising the preparation of the histologic material, 2) make photomicrographs of the histologic material for subsequent correlation with ancillary data, 3) after learning the technique of equating tubular involvement to testicular mass, to document said changes for full evaluation. This technician will spend full-time on this project.

At the present time we have in our employ three full-time technicians trained in various duties that will bear on this project. They will be spending varying periods of time on this project, but it is difficult to estimate the percentage thereof.

## VII. RELATED FINANCIAL ASSISTANCE

Although this is a University of Washington project, Dr. Roesch will be involved in the basic physics of this work. Before we can expose the inmate volunteers to neutron radiation, these basic studies will be carried out in his laboratory at Hanford. Therefore, Mr. Parker, the head of the radiation laboratory at Hanford, is writing a letter to the AEC in Richland. This will request their endorsement of Dr. Roesch's participation in this project. The AEC in Richland will then notify your office regarding this aspect.

Additional financial support of Dr. Paulsen's research activities is listed below:

NIH AM 05436-(2S1 End: Pituitary-Gonadal Interrelationship,  
\$22,145; 9/1/62 to 8/31/63

NIH T1 AM 5161-04: Endocrinology and Metabolism Reproductive  
Physiology (Training Grant); \$29,051; 7/1/63 to 6/30/64

## VIII. MATERIALS, EQUIPMENT AND FACILITIES

### A. Equipment and Facilities Available

1. Phase II will be carried out at Hanford with the entire facilities of Dr. Roesch's laboratory available to this project.

2. Permanent equipment, Endocrine Research Laboratory, University Division at USPHS Hospital, Seattle, Washington (Dr. Paulsen):

- a. Rat cages, battery
- b. Rabbit cages, battery
- c. Air-water separator, Sprague
- d. Extracorporeal circulation

- g. Coldroom (4°C), walk-in, work-in, pre-fab
- h. Spectrophotometer with voltage regulator, Beckman DB
- i. pH meter, Beckman Zeromatic
- j. Oven, drying, Despatch DO-7
- k. Microscope, Zeiss, binocular
- l. Microtome, American Optical 820
- m. Incubator, Thelco model 6
- n. Shaker, Aloe
- o. Water bath, Precision Scientific
- p. Typewriter, IBM electric, Executive
- q. Stenocord dictating machine
- r. Calculator, Friden SBQ
- s. Oven, paraffin, Boekel
- t. Bath, paraffin, National Appliance 8750
- u. Balance torsion, Roller-Smith
- v. Refrigerator, Astral, 4 cu'
- w. Refrigerator, G. E., 9 cu'
- x. Freezer, Amana
- y. Powerpack, Spinco Duostat RD-2
- z. Electrophoresis chamber, lucite, 20" x 20" x 7"
- aa. Photocopy equipment, Apeco
- bb. Microscope, Zeiss, with trinoc body
- cc. Camera Stand, Aristophot, with bellows camera and polaroid film holder
- dd. Tele-thermometer, YSI, with thermistory probes

B. Justification of equipment to be procured

1. Neutron Generator: This apparatus is an integral part of our program since it will be our neutron source. The particular generator selected has all the desired features for the work described above.

2. Shielding: This is necessary for the safe use of the neutron generator. It will be constructed at Hanford and later transported to the State Penitentiary.

3. Monitoring Instrumentation: This item is for controlling the dose delivered during an irradiation.

4. Dosimetry Instrumentation: This item is for measuring the dose delivered during an irradiation.

5. Operating Supplies: Items such as liquid nitrogen, deuterium gas, generator targets, etc., will also be required.

6. Remodeling Expenses:

a. USPHS Hospital, Seattle. The administration has set aside a large room for conversion into a histology laboratory. Our tissue culture operations have increased so that additional laboratory space is required for the microscopic work.

b. Walla Walla State Penitentiary. The warden has set aside a basement room in the hospital building for our use. This room will house the neutron generator and the remainder of our equipment. It will be necessary to provide adequate water, drainage and electrical facilities.

**IX. TRAVEL AND OTHER ITEMS**

Integral to the pursuit of our research project is the proposed travel. It is estimated that it will be necessary for Dr. Roesch to make approximately six trips to Seattle during the first year for the purpose of co-ordinating his aspect of the work with ours.

In addition, it is estimated that the principal investigator and one assistant will need to make approximately twenty trips to the prison at Walla Walla. This is for the purpose of interviewing inmates, exposing the inmates to the various doses of irradiation (under Dr. Roesch's supervision), performing testicular biopsies, arranging for urine collections, and in general supervising those details necessary for this research endeavor.

The necessity for procuring the listed items for the neutron source are evident and represent in Dr. Roesch's opinion our proper needs.

**X. BUDGET**

**A. Personnel**

1. Professional: None requested

2. Non-Professional:

Research technician (to be selected)	8052.00
Research secretary (half-time)	1854.00

**B. Stipends for Inmates:** 5000.00

**C. Equipment, Permanent:**

Neutron generator, Kaman Nuclear Model A-1000	20000.00
Beam deflection pulser, Model A-1100	1750.00
Shield for above (to be designed and constructed at Hanford)	3000.00
Monitor, BF 3 proportional counter, Nuclear Chicago NC-251	110.00
Voltage supply, amplifier, and scaler - Radiation Instrument Development Lab., Modular components 29-1, 30-19, 40-9 and 49-26	1530.00
Dosimeter - Thermoluminescent neutron dosimeter Controls for Radiation, Inc.	5000.00
Tissue culture hood	200.00
Incubator, Model #4	250.00
Telethermometer with probes	350.00
Centrifuge, HN with #815 head	400.00
Research microscope, Zeiss GFL	1200.00
Drying oven, VP 31472	125.00

**D. Travel:**

Seattle - Pasco, round-trip, air	166.43
Seattle - Walla Walla, round-trip, air	1474.41

E. Consumable Supplies:

Reagents, glassware, stains, media, etc. for histology  
and tissue culture 2000.00

F. Miscellaneous:

Cost of transporting urine specimens from Walla Walla to  
research laboratories in Seattle via Railway Express 1000.00  
Remodeling laboratory space at the USPHS Hospital in  
Seattle and Room B01 in the basement of the hospital  
building at the Walla Walla State Penitentiary 4000.00  
Cost of leasing the radium and construction of plastic  
mold 1000.00

G. Overhead (19% of salaries) 1882.14

TOTAL BUDGET \$60,364.02

Alternate Budget

Items A, B, C, D, E, G remain the same 54364.02

F. Miscellaneous:

Cost of transporting urine specimens 1000.00  
Remodeling laboratory space at the USPHS Hospital  
and Room B01 at the State Penitentiary 4000.00  
G.E. Maximar 250 KV, type 3 - monthly lease 348.50  
12 months 4182.00  
covers all maintenance, labor parts, installation  
Shipping expense 500.00

TOTAL BUDGET \$64,046.02  
(Alternate)

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C. Alvin Paulsen, M. D.  
Principal Investigator

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