



UNITED STATES
ATOMIC ENERGY COMMISSION

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OAK RIDGE OPERATIONS
P.O. BOX E
OAK RIDGE, TENNESSEE 37830

Oak Ridge Operations
REPOSITORY *Records Holding Area*
COLLECTION *Documents 1044-1994*
BOX No. *H-93-17 Bldg 2714-H*
FOLDER *Cont 4002*
Alexas CA 2-22-72

FEB 22 1972

Ralph Elson, Director, Contract Division

REQUEST FOR CONTRACT ACTION

It is requested that you take the necessary steps to process the following described contract action (CA):

1. Nature of Action Requested:

- Selection of New Contractor and/or Negotiation of Contract
Number:
Contractor:
- Modification of Contract
Number: **AT-(40-1)-4002**
Contractor: **Texas Tech University**

2. Nature of Services To Be Covered by Contract: Research

Title: **"The Effects of X-Irradiation on the Human Testis: An Electron Microscopic Cytochemical Study"**

3. Type of Contract:

- Support Agreement
- Cost Type
- Other

4. Amount of AEC Funds To Be Obligated by this CA: **\$9,000**

5. AEC Percentage of Est. Total Cost To Be Shown by this CA: **75%**

6. Description of Other Changes To Be Covered by this CA:

FEB 22 1972

Modify the contract to provide for the performance of additional research to be completed during the period March 1, 1972 through February 28, 1973. Increase the AEC Cumulative Support Ceiling from \$43,304 to \$52,304. Title to equipment shall vest in the Contractor under authority of AEC Act of 1954.

VE
2-22-72

7. Authority:

Form AEC-481 (CA) frm J. R. Totter, HQ,
dtd 2/7/72

OLE:JDB

OLA
CS Shw
2-22-72
Herman M Roth
Herman M. Roth, Director
Laboratory and University Division

CONTRACTS - 4002 (Texas Tech)
C.A.

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APPENDIX "A"

For the Contract Period March 1, 1972 through August 31, 1972.

ARTICLE A-I RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will conduct an electron microscope cytochemical examination of tissues from normal human testis materials which have received X-irradiation in the dose ranges of 20, 75, 200, and 600 rad. Samples will be obtained from a few minutes to several months following irradiation, to define the enzymic localizations that occur during a number of phases, i.e., spermatogonial degeneration and the recovery process when the seminiferous tubules are repopulated with the spermatogenic cell series. Location of enzymes will be compared in the irradiated material with the situation in the normal tissues. Enzymes to be examined include acid phosphatase, thiamine pyrophosphatase, glucose-6-phosphatase, inosine diphosphatase, and various other nucleoside di- and triphosphatases. Effects of irradiation on subcellular structures will also be studied.

The Principal Investigator, Dr. Jerry D. Berlin, expects to devote approximately 20% of his time or effort to the project.

ARTICLE A-II WAYS AND MEANS OF PERFORMANCE(a) Items Included in Total Estimated Cost:

(1) <u>Salaries and Wages:</u>	\$5,653
Principal Investigator 4 Research Assistants	
(2) <u>Employee Benefits:</u>	315
(3) <u>Supplies and Materials:</u>	1,559
Chemicals, glassware, electron microscope plates, photographic supplies, diamond knives, etc.	
(4) <u>Equipment to be Purchased or Fabricated by the Contractor:</u>	0
<u>a</u> Equipment Estimated to Cost Less than \$1,000:	
None	
<u>b</u> Equipment Estimated to Cost in Excess of \$1,000:	
None	
(5) <u>Travel:</u>	350
(6) <u>Publications and Communications:</u>	350

- (7) Electron Microscope Service Contract: \$1,600
- (8) Indirect Costs(Fixed at 37.6% of Salaries & Wages): 2,126
- (b) Items, if any, Singificant to the Performance of this Contract, but Excluded from Computation of Support Cost and from Consideration in Proportioning Costs:
- (1) Items to be Contributed by the Contractor:
None
- (2) Items to be Contributed by the Government:
None
- (c) Time or Effort of Principal Investigator(s) Contributed by Contractor, but Excluded from Computation of Support Cost and from Consideration in Proportioning Costs:
None under this paragraph

ARTICLE A-III The total estimated cost of items under A-II (a) above for the contract period stated in this Appendix "A" is \$11,953; the Commission will pay 75% of the actual costs of these items incurred during the contract period stated in this Appendix "A", subject to the provisions of Article III and Article B-XXVII. The estimated AEC Support Cost for the contract period stated in this Appendix "A" is \$9,000.

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U. S. ATOMIC ENERGY COMMISSION CONTRACT AUTHORIZATION		1. DATE FEB 7 1972	2. AUTHORIZATION NO. BM-72-402
3.A. TO Robert J. Hart, Manager Oak Ridge Operations Office		3.B. FROM John R. Totter, Director Division of Biology and Medicine	
4.A. CONTRACTOR (Name, Address, Department, etc.) TEXAS TECH UNIVERSITY Lubbock, Texas		4.B. PRINCIPAL INVESTIGATOR(S) JERRY D. BERLIN	
5. <input checked="" type="checkbox"/> NEW CONTRACT <input checked="" type="checkbox"/> RENEWAL <input type="checkbox"/> OTHER		6. TERM OF CONTRACT 3/1/72 thru 8/31/72	
7. CONTRACT NUMBER AT(40-1)-4002		8. RECOMMENDED TYPE OF CONTRACT: <input type="checkbox"/> FIXED PRICE <input type="checkbox"/> OTHER <input type="checkbox"/> COST REIMBURSEMENT <input checked="" type="checkbox"/> SPECIAL RESEARCH SUPPORT AGREEMENT (SRSA)	
9. EQUIPMENT TITLE TO VEST IN: <input type="checkbox"/> AEC <input checked="" type="checkbox"/> CONTRACTOR		10. SECURITY CLASSIFICATION: Work to be performed is under category I as defined by AEC Manual Appendix 3401.	
11. PROJECT TITLE THE EFFECTS OF X-IRRADIATION ON THE HUMAN TESTIS: AN ELECTRON MICROSCOPIC CYTOCHEMICAL STUDY			
12. HEADQUARTERS TECHNICAL CONTACT Frank T. Brooks, D.V.M.			
13. FINANCING			
A. OPERATING EXPENSES			
New AEC Funds		\$ 9,000	
Estimated AEC Balance From Prior Term, if any		\$	
.....		\$ 9,000	
Estimated Contractor Contribution, On Proportionate Sharing Basis, if any		\$ *	
Estimated Project Cost, For Pertinent Budget Period		\$	
Budget and Reporting Classification: 06 02 01			
Allotment Transfer: 06-21-91(24)			
B. PLANT AND CAPITAL EQUIPMENT\$			
Budget and Reporting Classification:			
Allotment Transfer:			
14. SPECIAL PROVISIONS AND INSTRUCTIONS:			
The technical aspects of the proposed work have been reviewed and are approved. A need currently exists for the results of the research or other work that is to be undertaken. None of the AEC funds shall be used to confer a fellowship.			
Please keep us informed as to any problems encountered in your negotiations, as well as the date of execution of this contract and the amount of funds obligated. If the budget as negotiated differs substantially from that in the proposal, please forward a copy of the revised budget to Headquarters.			
If not already submitted, a 200-word summary of the proposed work should be forwarded by the contractor as soon as possible after negotiation of the contract.			
* To be determined			
Note: This contract has been approved for 12 months with funds provided by this authorization for six months. Funds for the subsequent contract period will be issued in FY73 prior to August 31, 1972.			
15. SCOPE OF WORK			
A cytochemical study of the effects of acute irradiation on human spermatogenesis.			

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D 928
 FEB 8 1972
 CONTRACTS - 4002 (Texas Tech)
 C.A.

FEB 7 1972

Dr. Jerry D. Berlin
Texas Tech University
Department of Biology
Lubbock, Texas 79409

Dear Dr. Berlin:

I wish to inform you that Atomic Energy Commission contribution toward support of your project, "The Effects of X-Irradiation on the Human Testis: An Electron Microscopic Cytochemical Study," has been approved but at less than the requested level.

The negotiation of the contract, including financial detail, will be handled by our Oak Ridge Operations Office, and you may expect to hear from a representative of that office shortly. Responsibility for the technical and scientific administration will, of course, remain with the Division of Biology and Medicine in Washington.

We wish you every success in your work.

Sincerely,

Frank T. Brooks, D.V.M.
Medical Research Branch
Division of Biology and Medicine

Bcc: Oak Ridge Operations Office ✓

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FEB 8 1972

1. Budget: From March 1, 1972 to August 31, 1972

	<u>Estimated Requirements</u>	
	<u>Texas Tech University</u>	<u>Atomic Energy Commission</u>
1. Salaries and Wages		
a. Principal Investigator, J. Berlin, Associate Prof. \$1711 per month (1/5 time for 6 months)	\$ 2,053.00	
b. Graduate Assistants		
Graduate Assist. (1/2 time for 3 months)	-	\$ 810.00
Graduate Assist. (1/2 time for 3 months)		810.00
Graduate Assist. (full-time for 3 months)		990.00
Graduate Assist. (full-time for 3 months)		990.00
c. Fringe Benefits (OASI: 5.2% of salaries: Group Insurance for J. Berlin at \$3.50 per man month)	128.00	187.00
2. Supplies and Materials		
a. Chemicals		359.00
b. Glassware		150.00
c. Electron Microscope Plates, other Materials		300.00
d. Other photographic supplies		200.00
e. Resharpen diamond knives		500.00
f. Office supplies and miscellaneous		50.00
3. Capital Equipment: None Requested for 3rd Year		
4. Publication Costs		300.00
5. Communication		50.00
6. Travel		
to Seattle, Washington		350.00
7. Hitachi Electron Microscope Service Contract		1,600.00
8. Indirect Charges 37.6% of salaries and wages in accordance with policy established with DOD TDS 66-67	<u>772.00</u>	<u>1,354.00</u>
TOTAL PROJECT COST		\$11,953.00
Percentage and Amount Contributed by Texas Tech University 24.7%		2,953.00
Percentage and Amount Requested from the AEC 75.3%		9,000.00

1034842

Texas Tech University

Office of Research Services
P.O. Box 4670

February 15, 1972

Lubbock, Texas 79409
Phone (806) 742-6247



Mr. C. S. Shoup, Chief
Research Contracts Branch
Laboratory and University Division
U. S. Atomic Energy Commission
Oak Ridge Operations
P.O. Box E
Oak Ridge, Tennessee 37830

RE: Contract No. AT-(40-1)-4002.

Dear Mr. Shoup:

Enclosed please find six copies of the revised budget request for the renewal of the above referenced contract. This revised budget is based on both the AEC Support of \$9,000 for the six months period and the support to be contributed by the University.

If we can be of any service or assistance, please call on us.

Sincerely,


Fred E. Briggs
Director

FEB;sw

Enclosures (6) *OK*

cc: Comptroller
Dr. Berlin

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11 1169
FEB 18 1972
CONTRACTS - 4002 (Texas Texas)
C.A.

FEB 10 1972

Dr. Jerry D. Berlin
Department of Biology
Texas Tech University
Lubbock, Texas 79409

CONTRACT NO. AT-(40-1)-4002

Dear Dr. Berlin:

Please refer to my letter of February 8, 1972, concerning the renewal of the above contract.

We have received written authorization from AEC Headquarters to extend the period under the contract from March 1, 1972 through February 28, 1973. However, due to the shortage of FY 1972 funds, we have been authorized to fund only the first six months of the contract period and at a level not to exceed \$9,000. We understand that an additional \$9,000 will be made available for the remaining six months, sometime after July 1972. Of course, the additional funding will be contingent upon the availability of FY 1973 funds.

Please provide us with a revised budget based on both the AEC Support of \$9,000 for the six months period and the support to be contributed by the University. Although the proposed modification to the contract will contain a budget for six months only, we plan to revise the budget if and when the additional funds are received to cover a one year period.

Please submit a revised budget to this office at an early date. If you have any questions concerning the proposed modification or the AEC funding, please let us know.

Sincerely,

ORIGINAL SIGNED BY
JAMES W. NEHL

ACTING

C. S. Shoup, Chief
Research Contracts Branch
Laboratory and University Division

X 859

OLE:JDB

OFFICE ▶	Adm. Ser. Br. M. L. Pennington, TTU	Res. Cont. Br.			
SURNAME ▶	Burleson:00	Nehls		CONTRACTS - 4002 (Texas Texas)	
DATE ▶	2-9-72	2/10			C.A.

1034844

Record copy



UNITED STATES
ATOMIC ENERGY COMMISSION

OAK RIDGE OPERATIONS
P.O. BOX E
OAK RIDGE, TENNESSEE 37830

AREA CODE 615
TELEPHONE 483-8611

August 17, 1971

John R. Totter, Director, Division of Biology and Medicine, HQ

RENEWAL OF CONTRACT NO. AT-(40-1)-4002 - TEXAS TECH UNIVERSITY

We are submitting for your review and appropriate action the following information concerning the contract which will expire on **November 14, 1971**:

- 1. Renewal Proposal (4)
- 2. Progress Report (4)
- 3. Financial Statement (4)
- 4. 200-Word Summary (3)

We shall appreciate your advising us of your decision so that we may proceed with the necessary contract action at the earliest possible date.

Herman M. Roth
Herman M. Roth, Director
Laboratory and University Division
Oak Ridge Operations

Y 4912

OLE: **OMO**

Enclosures:
As Listed Above

BC: D. S. Zachry, w/2 cys. Progress Report & Form AEC-427
C. S. Shoup, w/cys. Encl.
Alice Brown, w/o Encl.

Adm. Ser. Br.
omo 8/17
Osborne
8-17-71

AUG 17 1971

CONTRACTS - 4002 (Texas Tech C.A.)

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Texas Tech University

Office of Research Services
P.O. Box 4670

August 12, 1971

Lubbock, Texas 79409
Phone (806) 742-6247

Dr. C. S. Shoup, Chief
Research Contracts Branch
Laboratory and University Division
United States Atomic Energy Commission
Oak Ridge Operations
P. O. Box E
Oak Ridge, Tennessee 37830

Dear Dr. Shoup:

Enclosed are seven copies of a request for continuing support of a research project entitled, "The Effects of X-irradiation on the Human Testis: An Electron Microscopic Cytochemical Study." This proposal has been developed by Dr. Jerry D. Berlin of the Department of Biology. Funds in the amount of \$18,980 are requested for this program which is proposed to begin November 15, 1971.

Also enclosed are seven copies of the Progress Report for this project for the period November 15, 1970 through November 14, 1971, along with seven copies of the Financial Statement for the period November 15, 1970 through July 31, 1971.

Your favorable consideration of this proposal will be greatly appreciated.

Sincerely,


Fredy E. Briggs
Director

FEB:jb
Enclosures *OK*
cc: Comptroller
Graduate School
Dr. Jerry D. Berlin

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CONTRACTS - 4002 (Texas Tech) C 6758
AUG 16 1971 C.A.

Texas Tech University

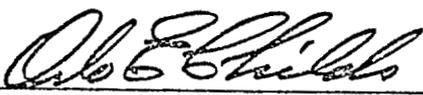
Office of Research Services
P.O. Box 4670

Lubbock, Texas 79409
Phone (806) 742-6247

FINANCIAL STATEMENT FOR
THE UNITED STATES ATOMIC ENERGY COMMISSION
CONTRACT NO. AT-(40-1)-4002

1. Total actual project cost to date for the current period	<u>\$13,737.76</u>
2. Estimated total cost for remainder of period	<u>\$12,355.40</u>
3. Total actual and estimated cost chargeable to AEC for current period based on percentage of cost agreed upon as contained in A-III of Appendix "A" to contract	<u>\$20,920.12</u> ²¹³⁹⁶ = 822
4. Accumulated costs chargeable to AEC (include costs reported in certified statement for preceding period(s) and the costs stated in Item 3 above)	<u>\$43,304.00</u>
5. Accumulated AEC Support Ceiling as stated in Article III of contract	<u>\$43,304.00</u>
6. Total estimated AEC funds remaining under contract (Subtract Item 4 from Item 5) which may be used to reduce amount of new funds required from AEC for proposed renewal period.	<u>\$ -0-</u>

Endorsement:



Vice President for Research and Special Programs

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PUBLICATION BY AEC AUTHORIZED

NOTICE OF RESEARCH PROJECT SCIENCE INFORMATION EXCHANGE SMITHSONIAN INSTITUTION

U.S. ATOMIC ENERGY COMMISSION

SIE NO.

AEC CONTRACT NO. AT-(40-1)-4002

SUPPORTING DIV. OR OFFICE: NAME & ADDRESS OF CONTRACTOR OR INSTITUTION: (State the division, department, or professional school, medical, graduate or other, with which this project should be identified.)

Department of Biology Texas Tech University Lubbock, Texas 79409

TITLE OF PROJECT: The Effects of X-irradiation on the Human Testis: An Electron Microscopic Cytochemical Study

NAMES, DEPARTMENT, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS AND OTHER PROFESSIONAL SCIENTIFIC PERSONNEL: (not including graduate students) engaged on the project, and fraction of man-year devoted to the project by each person.

Jerry D. Berlin, Ph.D. Associate Professor and Principal Investigator 1/3 man year

NO. OF GRADUATE STUDENTS ON PROJECT: 2 NO. OF GRADUATE STUDENT MAN-YEARS: 1 1/4

SUMMARY OF PROPOSED WORK: (200-300 words, omit Confidential Data). Summaries are exchanged with government and private agencies supporting research, are supplied to investigators upon request, and may be published in AEC documents. Make summaries substantive, giving initially and for each annual revision the following: OBJECTIVE; SCIENTIFIC BACKGROUND FOR STUDY; PROPOSED PROCEDURE; TEST OBJECTS AND AGENTS. Normal human testes which have received X-irradiation doses of 20, 75, 200, and 600 r will be subjected to an electron microscopic cytochemical examination to determine the effects of irradiation on the subcellular structure and function of the testis. The testis is an extremely radiosensitive tissue. An early irradiation effect is the breakdown of spermatogonial cells which leads to azoospermia. The reappearance of the spermatogenic cell series marks the beginning of the recovery process, however, the timing of complete repopulation of the seminiferous tubules is dose dependent and may require several years for high doses. In addition to these morphological events, it has been suggested that irradiation may alter the localization of various enzymes. We will examine biopsies obtained from a few minutes to several months after irradiation to define changes that occur in the subcellular structure and in enzymic localizations during spermatogonial degeneration and the recovery process. Our results with the irradiated material will be compared with that we have found to occur in normal, non-irradiated human testes. The enzymes to be examined include acid phosphatase, thiamine pyrophosphatase, glucose-6-phosphatase, inosine diphosphatase, and various other nucleoside di- and triphosphatases.

RESULTS TO DATE: Shortly after X-irradiation of human testes, spermatogonia degenerate and are phagocytised by Sertoli cells. Other radiation-induced morphological changes include alterations in mitochondrial cristae of spermatogenic cells and altered acrosomal structure. Irradiation results in increased acid phosphatase activity in Sertoli cells and a decrease in thiamine pyrophosphatase and inosine diphosphatase activity, a dislocation of glucose-6-phosphatase activity, and no change in the activities of adenosine monophosphatase or adenosine triphosphatase in all of the cells within the seminiferous tubules.

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Table with 2 columns: BUDGET (PRIMARY, SECONDARY) and PROGRAM CATEGORY NO.

Signature of Principal Investigator: Jerry D. Berlin DATE: Aug 11, 1971

RENEWAL PROPOSAL FOR
THE UNITED STATES ATOMIC ENERGY COMMISSION
SUPPORT OF RESEARCH

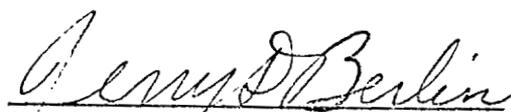
Project Title: The Effects of X-irradiation on the Human Testis: An
Electron Microscopic Cytochemical Study.

Desired Starting Date: November 15, 1971

Institution: Texas Tech University
Lubbock, Texas 79409

Principal Investigator: Jerry D. Berlin
Associate Professor
Department of Biology

Authentication:


Jerry D. Berlin
Principal Investigator


Dr. Orlo E. Childs
Vice President for Research

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~~C 6758~~
AUG 16 1971

PROJECT ABSTRACT

Normal human testes that have received X-irradiation doses of 20, 78, 200, and 600 r will be subjected to an electron microscopic cytochemical examination. Samples will be obtained from 6 months to 6 years postexposure to define enzymic localizations that occur during the depleted state, i.e., the azoospermic condition, and the recovery process when the seminiferous tubules are repopulated with the spermatogenic cell series. In particular, the totipotency of the few spermatogonia that remain in the azoospermic testis will be determined with marker enzymes. The enzymes to be examined include thiamine pyrophosphatase, inosine diphosphatase, acid phosphatase, glucose-6-phosphatase, and adenosine mono- and triphosphatase. Information derived from this study should provide knowledge about the effects of X-irradiation on the structure and function of the human testis.

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A. Scientific Background:

The goal of this research is to determine the effect of X-irradiation on the human testes. To this end we have used electron microscopic cytochemistry to observe testicular ultrastructure and enzymic localization. The six enzymes that we have examined are thiamine pyrophosphatase, inosine diphosphatase, acid phosphatase, glucose-6-phosphatase, adenosine monophosphatase, and adenosine triphosphatase. To date, there have been two distinct phases of the program. Phase I consisted of determining the localizations of the above six enzymes in normal, human testes and was performed the first year of the project. For convenience, the cytochemistry of the control human testis is duplicated with minor corrections (addition of acid phosphatase activity in the Sertoli cell Golgi complex and adenosine triphosphatase activity to the plasma membrane of spermatids) from last year's proposal (Table I). Phase II, primarily conducted during the past year, has dealt with the early effects of irradiation on the human testis. Our results on morphology and enzymic localization during the first month postexposure will be subsequently discussed. Phase III, the work outlined in this proposal, concerns the morphology and cytochemistry of human testes in the depleted state and during the recovery stages.

Mammalian testes have been known to be radiosensitive since the studies of Albers-Schonberg (1903, Munch. Med. Wochschr. 50:1859-1860). The extreme sensitivity of the human testis was first reported two years later (Tilden, et al., 1905, as cited in Harvey, 1908, J. Path. Bact. 12:549-556). Radiation-induced azospermia was thought to result from a prolonged inhibition of spermatogonial mitosis (Eschenbrenner, et al., 1948, J. Nat. Cancer Inst. 9:133-147); however, Oakberg (1955, Radiation Res. 2:369-391; 1955, J. Morph.

TABLE I
CYTOCHEMISTRY OF CONTROL HUMAN TESTIS

	AcPase	G-6-Pase	AMPase	IDPase	ATPase	TPPase
<u>Sertoli Cells</u>						
Nuclear envelope				±		
Endoplasmic reticulum		+		+		+
Golgi complex	+			+		
Lysosomes	+					
Lipofuscin bodies	+		+	-	+	
Plasma membrane						
<u>Spermatogonia through Secondary Spermatocytes</u>						
Nuclear envelope				±		
Endoplasmic reticulum				+		+
Golgi complex				+		
Cytoplasmic vesicles			+	-	+	
Plasma membrane						
<u>Spermatids</u>						
Acrosome				±		+
Plasma membrane						

+ = strong reaction; ± = weak reaction; - = no reaction.

Abbreviations: AcPase = acid phosphatase; G-6-Pase = glucose-6-phosphatase; AMPase = adenosine monophosphate; IDPase = Inosine diphosphatase; ATPase = adenosine triphosphatase; TPPase = thiamine pyrophosphatase.

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97:39-54) demonstrated that the characteristic azoospermic condition following radiation exposure was actually caused by spermatogonial destruction.

Our ultrastructural examination of the early effects of irradiation revealed spermatogonial degeneration and we feel that this phenomenon is probably the primary effect of irradiation on the testis; certainly it leads to azoospermia. However, in irradiated testis, we observed additional morphological alterations, e.g., a change in mitochondrial cristae of spermatogenic cells, vacuolate cytoplasm of spermatogenic cells, acrosomal alterations, and an increased frequency of spermatids with multiple heads. The significance of these structural modifications is unknown and we presently categorize them as subtle irradiation effects, although they are admittedly not very subtle in some cases.

We have examined approximately 55 irradiated human testes including samples taken from 15 minutes to 7 years postexposure. The bulk of these samples were irradiated with either 20 r, 78 r, 200 r, or 600 r and were biopsied during the first month postexposure (see accompanying Progress Report for details).

A brief summation of our cytochemical results on irradiated human testes during the first month postexposure reflects differing radiosensitivities of the enzymes examined. For example, acid phosphatase activity increases in the Sertoli cells and this increased activity appears to be directly related to spermatogonial degeneration and subsequent phagocytosis by Sertoli cells. Thiamine pyrophosphatase and inosine diphosphatase activity decreases in the spermatogenic cells, but remains unchanged in the Sertoli cells. The decreased activity of these two enzymes in the spermatogenic

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cells is strikingly different. At doses up to 600 r X-irradiation, the loss of inosine diphosphatase activity was time dependent and occurred between 17 and 24 days postexposure. The decrease in thiamine pyrophosphatase activity was both dose and time dependent. Low radiation doses of 20 and 78 r did not influence thiamine pyrophosphatase activity through at least 24 days following exposure. However, by 17 days after 200 and 600 r doses, we were unable to detect any activity for this enzyme in spermatogenic cells. Glucose-6-phosphatase activity appears to be decreased at short times postexposure to low doses of X-rays (11 days postexposure to 20r) and at longer times and higher doses (15 weeks postexposure to 200 r) is displaced from the rough endoplasmic reticulum of Sertoli cells. Finally, at the doses and times examined, we have been unable to find any change in localizations or activities of adenosine mono- or triphosphatase. Thus, the six enzymes that we have studied in irradiated human testes run the gamut from being radioresistant to exhibiting varying degrees of radiosensitivity, including increased activity, decreased activity, and/or altered localization. We feel that the increased acid phosphatase activity is related to the primary effect of irradiation on the testis, i.e., spermatogonial degeneration; however, the reduced activities of thiamine pyrophosphatase, inosine diphosphatase, and glucose-6-phosphatase could be termed subtle irradiation effects. At any rate, in addition to spermatogonial degeneration, X-irradiation can profoundly effect cellular metabolism without altering the structural integrity of associated organelles.

Pertinent to this proposal is information that has been obtained on the depleted state and the recovery process via light microscopy in Dr. Carl Heller's laboratory (1971, Personal Communication). Type B spermatogonia

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degenerate immediately after irradiation. Type A spermatogonia may be observed as late as 1 to 2 months postexposure, but their numbers reach an extremely low level at approximately 2 months postexposure with radiation doses sufficient to induce azoospermia. At approximately 6-7 months postexposure, spermatogonia begin to reappear in all radiation doses examined. Hence, it appears that recovery is initiated at approximately 200 days following irradiation regardless of the radiation dose; however, the rate of recovery is dose dependent. For example, complete recovery for 600 r has yet to be observed, but is thought to require 6-7 years. At 200 r, complete recovery has been observed in 2-3 years, and with 20 and 78 r complete recovery is found to occur within one year.

Several questions are posed by the above information and by our cytochemical and morphological studies of the early effects of irradiation. For example, why do the spermatogonia remaining in the testis after irradiation remain latent instead of immediately initiating recovery? Why is the reappearance of spermatogonia at 200 days postexposure not dose dependent whereas the rate of recovery is dose dependent? Are these two questions related to the subtle effects of irradiation that we have previously described? It is possible that our examination of marker enzymes will enable us to evaluate the totipotency of spermatogonia during the recovery process. We propose to examine the cytochemistry of the depleted testis and of the recovering testis for enzymic as well as morphological recovery to determine the importance of subtle effects on the recovery process. It must be emphasized that the rarity of spermatogonia in the irradiated testis negates their ever being sufficiently concentrated for biochemical or physiological

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examination. Thus, it may remain for cytochemical studies to answer the enigma of testicular recovery following irradiation.

Finally, it should be mentioned that cytochemical differences exist between human testis and testes of other animals. Specifically, we have found localization sites for thiamine pyrophosphatase, inosine diphosphatase, adenosine triphosphatase, and glucose-6-phosphatase to be different than that reported for the rat (Tice and Barrnett, 1963, Anat. Rec. 147: 43-64; Reissenweber, 1970, Histochemie 21:73-83) and the Chinese hamster (Chang, Yokoama, and Brinkley, 1970, J. Cell Biol. 47:33a). As pointed out in our original proposal, other differences occur in testicular morphology and radiation sensitivity between man and other species. All of these things make extrapolation of information from other animals to man exceedingly difficult. Hence, a suitable substitute for human tissues does not exist, and if one is interested in resolving problems in man, then one must examine man. Although the work outlined in this proposal will use human tissues, it must be emphasized that no additional manipulation of human subjects will be required; we are simply seeking to take advantage of what will be existing experimental material.

B. Scientific Scope:

The objective of this study is to define changes in ultrastructure and enzyme localization that occur after X-irradiation of human testes. Emphasis will be placed on examination of testes in the depleted state and during the recovery process. Specifically, we hope to determine, by marker enzymes, the totipotency of the spermatogonia that occur in the testis after irradiation, but prior to repopulation. We anticipate that enzymic recovery within these spermatogonia may occur prior to, and signal the beginning of, repopulation of the spermatogenic cell series. Additionally, we will examine

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the dose dependent recovery process and attempt to establish the importance of enzymic recovery on repopulation of the spermatogenic cells after radiation-induced azoospermia. This work is designed to extend to the subcellular level our present knowledge about the effects of X-irradiation on the human testis. The Principal Investigator is unaware of any comparable work in progress in any other laboratory.

C. Experimental Design:

We will continue to receive material for this study through the courtesy of Dr. Carl Heller, Director, Division of Reproductive Physiology, Pacific Northwest Research Foundation, Seattle, Washington. The human testicular tissue is obtained as part of Dr. Heller's program from volunteer inmates in the Oregon State Prison, Salem, Oregon. Dr. Heller's program projections are such that the material required for this study will be available during the normal course of his work, and experimental manipulation of human subjects will not be duplicated for this study.

Tissue samples will be available in a variety of times following exposure to 20, 78, 200, and 600 r X-irradiation. Program projections now call for no additional irradiations; however, biopsies will be obtained from previously irradiated individuals for follow-up studies. Material from the last testicular irradiation will be in the initial phases of the recovery process by the end of 1971. Therefore, material studied during the time proposed for the present project will be in the terminal stages of the depleted state as well as in various stages of recovery. More precisely, for the dates covered by this proposal, we will be receiving biopsies of 600 r irradiated tissues from 6 to 48 months postexposure, of 200 r tissues from 6 to 70 months postexposure, of 78 r tissues from 6 to 25 months postexposure, and of 20 r tissues from 6 to 25 months postexposure.

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Biopsied testicular tissue will be initially fixed at the prison by a technician from Dr. Heller's laboratory in Seattle. As outlined in our original proposal, to simplify accounting procedures, Dr. Heller's laboratory will be submitting a separate budget to cover the costs incurred by the technician in processing the samples at the prison. Because of our success to date, the handling of tissues at the prison will remain essentially identical to that described in our original proposal.

When we receive the samples the tissues will be processed for the following marker enzymes: thiamine pyrophosphatase, inosine diphosphatase, acid phosphatase, glucose-6-phosphatase, and adenosine mono- and triphosphatase. The preparations will be made in a routine manner for electron microscopic cytochemistry with the steps including a buffer wash to eliminate the fixative, sectioning the tissue at 40 micra with a Smith-Farquhar copper, incubating the sections in media containing appropriate substrate, a buffer wash, fixation in osmium tetroxide, ethanol dehydration, and embedding in either Epon or a new low viscosity plastic medium. Stained and unstained thin sections will then be examined and photographed in an electron microscope.

D. Effect of Program of Graduate Education:

This program will continue to have an important role in the development of the Principal Investigator's graduate program at Texas Tech University by providing training for graduate students with a fairly unique tissue and with the technique of electron microscopic cytochemistry. One of the Graduate Assistants on the program has already presented a paper at a national meeting (American Society for Cell Biology, November 1970, San Diego; see Progress Report for abstract) and is in the process of preparing

a paper for publication. The other Graduate Assistant is in the terminal stages of manuscript preparation. Hence, the program is directly benefiting the graduate education of these students. Needless to say, this activity is recognized by the other graduate students and, as an indirect result, the program is raising the productivity of our entire graduate program.

E. Scientific Personnel:

1. Principal Investigator: Jerry D. Berlin, Ph.D.

Present Position: Associate Professor

Fraction of Time of Project:

Academic Year: 20%

Summer Months: 50%

Social Security Number: 490-34-8036

Scientific Experience:

Dr. Berlin was a Senior Research Scientist in the Biology Department at Hanford Laboratories (operated by Battelle Memorial Institute for the Atomic Energy Commission), Richland, Washington for four years (September 1964 to August 1968). During that time he directed the Electron Microscope Laboratory and obtained considerable experience with the subcellular effects of radiation injury and with the technique of electron microscopic cytochemistry. During the tenure of that employment, an electron microscopic study of human spermatogenic tissue was initiated with Dr. Carl Heller, Director of Reproductive Physiology, Pacific Northwest Research Foundation, Seattle, Washington. The proposed work is a continuation of that study via an Atomic Energy Commission Contract presently in effect. Dr. Berlin has been an Associate Professor of Biology at Texas Tech University since September, 1968.

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Publications:

- Bowen, C. C., G. A. Peyton, and J. D. Berlin. 1963. The fine structure of the host-parasite interface of some obligate fungal parasites. *Amer. J. Botany* 50:624-625.
- Berlin, J. D. and C. C. Bowen. 1964. The host-parasite interface of Albugo candida on Raphanus sativus. *Amer. J. Botany* 51:445-452.
- Berlin, J. D. and C. C. Bowen. 1964. Centrioles in the fungus Albugo candida. *Amer. J. Botany* 51:650-652.
- Berlin, J. D. and C. C. Bowen. 1965. Mitosis and zoospores formation in Albugo. *Amer. J. Botany* 52:613.
- Dean, J. M. and J. D. Berlin. 1965. The effect of temperature acclimation on the tissues of Salmo gairdneri (rainbow trout). *Amer. Zoologist* 5:696.
- Berlin, J. D. and J. M. Dean. 1966. Temperature-induced alterations in rainbow trout hepatocyte structure and function. *J. Cell Biol.* 31:12A.
- Berlin, J. D. 1967. The localization of acid mucopolysaccharide in the Golgi complex of intestinal goblet cells. *J. Cell Biol.* 32:760-766.
- Berlin, J. D. and J. M. Dean. 1967. Temperature-induced alterations in hepatocyte structure of rainbow trout (Salmo gairdneri). *J. Exptl. Zoology* 164:117-132.
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- Berlin, J. D. 1968. The ultrastructural localization of acid mucopolysaccharides in the intestine after irradiation. *Radiation Res.* 34:347-356.
- Mahoney, T. D., J. D. Berlin, J. C. Pekas, and M. F. Sullivan. 1968. The effect of bile on the mucosa of the intestine. In: M. F. Sullivan (Ed.), *Gastrointestinal Radiation Injury*, pp. 42-56. Excerpta Medica Foundation, Amsterdam.
- Adee, R. R., C. L. Sanders, and J. D. Berlin. 1968. Subcellular localization and identification of alpha emitters by electron microscopic autoradiography. *Health Physics.* 14:461-463.
- Dean, J. M. and J. D. Berlin. 1969. Alterations in hepatocyte function of thermally acclimated rainbow trout (Salmo gairdneri). *J. Comp. Biochem. Physiol.* 29:307-312.
- Berlin, J. D. and J. C. Ramsey. 1970. Electron microscopy of the developing cotton fiber. In: C. J. Arceneaux (Ed.). *Proceedings of the 28th Annual Meeting, Electron Microscope Society of America*, Claitor's Publishing Division, Baton Rouge. pp. 128-129.

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- Berlin, J. D. and D. D. Mahlum. 1970. Comparative morphological and functional studies of neptunium-induced fatty livers in rats. *Lab. Invest.* 22:252-259.
- Vollet, J. J. and J. D. Berlin. 1970. An electron microscopic cytochemical study of the effects of X-irradiation on mammalian testes. *J. Cell Biol.* 47:217a.
- Rowley, M. J., J. D. Berlin, and C. G. Heller. 1971. The ultrastructure of four types of human spermatogonia. *Z. Zellforsch.* 112:139-157.
- Allenspach, A. L. and J. D. Berlin. ca. 1971. The Golgi complex in the esophageal mucous glands of the newly hatched chick. *J. Morph.* In Press.
- Brackeen, R. B. and J. D. Berlin. ca. 1971. An electron microscopic cytochemical study of the effects of X-irradiation on inosine diphosphatase and thiamine pyrophosphatase of the human testis. In Preparation.

Research Reports:

- Mahoney, T. D. and J. D. Berlin. 1966. Ultrastructural study of intestinal radiation damage. Pacific Northwest Laboratory Annual Report for 1965 in the Biological Sciences. BNWL-280 (Pacific Northwest Laboratory, Richland, Washington). pp. 10-11.
- Berlin, J. D. and J. M. Dean. 1966. Ultrastructural changes in fish livers associated with temperature acclimation. *Ibid.*, pp. 115-117.
- Berlin, J. D. 1967. Localization of acid mucopolysaccharides in the Golgi complex of intestinal goblet cells. Pacific Northwest Laboratory Annual Report for 1966 in the Biological Sciences. BNWL-480 (Pacific Northwest Laboratory, Richland, Washington), pp. 12-51.
- Berlin, J. D. and D. D. Mahlum. 1967. Neptunium-induced fatty livers in rats: Electron microscopic and biochemical studies. *Ibid.*, pp. 43-46.
- Dilley, J. V. and J. D. Berlin. 1967. Effect of chronic cyanide ingestion on lung collagen. *Ibid.*, pp. 81-83.
- Berlin, J. D. 1967. Temperature-induced differences in acid phosphatase levels of rainbow trout livers. *Ibid.*, 157-159.
- Berlin, J. D. 1968. Acid mucopolysaccharide localization in intestinal absorptive cells after irradiation. Pacific Northwest Laboratory Report for 1967 in the Biological Sciences. BNWL-714 (Pacific Northwest Laboratory, Richland, Washington), pp. 7.14-7.17.

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Berlin, J. D., C. G. Heller, and M. Rowley. 1970. Electron microscopy of human spermatogonia. Pacific Northwest Laboratory Report for 1968 in the Biological Sciences. BNWL-1050 (Pacific Northwest Laboratory, Richland, Washington), pp. 6.25-6.29.

2. Graduate Assistant: Steven S. Barham, B.S.
Graduate Student
Social Security Number: 464-82-1196

Fraction of Time on Project:

Academic Year: 50%

Summer Months: 100%

Scientific Experience: Research Assistant, January 1971 to present,
on program.

Publications: None

3. Graduate Assistant: To Be Named

Fraction of Time on Project:

Academic Year: 50%

Summer Months: 100%

F. Other Personnel: None

G. Other Financial Assistance:

From non-University sources on this project: None

From non-University sources on unrelated projects:

The Principal Investigator has a \$7,000 grant from Cotton, Incorporated for unrelated work that will terminate December 31, 1971.

H. Facilities:

The Department of Biology at Texas Tech University, where the work outlined in this proposal will be performed, has an Electron Microscope Laboratory of approximately 3600 square feet of floor space.

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Of this, approximately 1500 square feet is devoted to a teaching laboratory that contains a Hitachi model HS-8-2 electron microscope. This teaching complex has its own electron microscope room, plate and print darkrooms, a general preparation room, a microtomy room, and a teaching laboratory. The remaining 2100 square feet of floor space is allocated to a research complex that contains a Hitachi HU-11E-1 electron microscope and will continue to be available for this study. The research complex has a general preparation room, cold room, print darkroom, microtomy room, electron microscope room, plate darkroom, a room used specifically for cytochemistry, and office space for the Principal Investigator and Research Assistants. Included in this complex are the Reichert Om U2 ultramicrotome purchased with contract funds and a Reichert research light microscope purchased for this program with matching funds from Texas Tech University. All of the major pieces of equipment required for the successful completion of this study are already in place.

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I. Budget: From November 15, 1971 to November 14, 1972

	<u>Estimated Requirements</u>	
	<u>Texas Tech University</u>	<u>Atomic Energy Commission</u>
1. Salaries and Wages		
a. Principal Investigator, J. Berlin, Associate Prof., \$1678 per month (Full-time for 1 1/2 months) (1/5 time for 9 months)	\$ 3,020.00	\$ 2,517.00
b. Graduate Assistants		
Graduate Assist. (1/2 time for 9 months)		2,500.00
Graduate Assist. (1/2 time for 9 months)		2,700.00
Graduate Assist. (full-time for 3 months)		861.00
Graduate Assist. (full-time for 3 months)		900.00
c. Fringe Benefits (OASI: 5.2% of salaries; Group Insurance for J. Berlin at \$3.50 per man month).	164.00	498.00
2. Supplies and Materials		
a. Chemicals		640.00
b. Glassware		300.00
c. Electron Microscope Plates, other Materials		600.00
d. Other photographic supplies		400.00
e. Resharpen diamond knives		200.00
f. Office supplies and miscellaneous		50.00
3. Capital Equipment: None Requested for 3rd Year		
4. Publication Costs		600.00
5. Communication		50.00
6. Travel		
a. To Scientific Meeting		400.00
b. To Seattle, Washington and Salem, Oregon		600.00
7. Hitachi Electron Microscope Service Contract		1,600.00
8. Indirect Charges 37.6% of salaries and wages in accordance with policy established with DOD TDS 66-67	1,136.00	3,564.00
TOTAL PROJECT COST		\$23,300.00
Percentage and Amount Contributed by Texas Tech University 18.5		4,320.00
Percentage and Amount Requested from the AEC - 81.5		18,980.00

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