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RENEWAL PROPOSAL: Year 3

February 1976

REPETITIOUS NATURE OF REPAIRED DNA IN MAMMALIAN CELLS

U.S.E.R.D.A. Contract No. AT(40-1)-4761

June 1, 1975 - May 31, 1976

Submitted to:

Energy Research and Development Administration
Oak Ridge, Tennessee 37830

Submitted by:

Martin L. Meltz, Ph.D.
Principal Investigator

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Renewal Proposal: Year 3

U.S.E.R.D.A. Contract No. AT-(40-1)-4761

1. Title

REPETITIOUS NATURE OF REPAIRED DNA IN MAMMALIAN CELLS

Submitted by:

Martin L. Meltz, Ph.D.

Associate Foundation Scientist

2. Institution

Southwest Foundation for Research and Education

8848 West Commerce at Loop 410

P. O. Box 28147

San Antonio, Texas 78284

Martin L. Meltz, Ph.D.
Principal Investigator

February 27, 1976
Date

Edward F. Feith, Treasurer
Official Aurhorized to Sign for
Southwest Foundation for Research
and Education

February 27, 1976
Date

Project Period

June 1, 1976 - May 31, 1977

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3. Project Abstract

During the third year of this project, several specific and one broader investigation will be undertaken. The specific studies are based on results which we obtained in the first two years of this project, or which were described in our initial or renewal applications, and include: a) Reexamination of the kinetic reassociation of repair replicated DNA obtained from WIL_2-A3 cells upon methylmethanesulfonate treatment over the entire C_0t range. b) Examination of the kinetic reassociation of DNA of WI-38 cells undergoing repair replication at different times after UV or MMS treatment. c) Comparison of the extent of repair replication occurring in skin fibroblast cells from individuals of different ages grown in vitro and treated at equal passage numbers with UV or MMS, and if significant differences are observed, investigation of the kinetic reassociation of the isolated DNA to determine the distribution of repair replication radioactivity in DNA of different sequence frequencies. d) Comparison of the extent of repair occurring in human WIL_2-A3 lymphoblastoid cells and human WI-38 lung cells of embryonic origin, after UV exposure, to confirm the normality of the former. In addition, determination of the extent of repair upon treatment with a second chemical agent, 4-nitroquinoline-1-oxide. e) Induction of AHH in WI-38 cells in vitro, and attempt to detect whether a greater amount of repair occurs during benz(α)pyrene treatment in induced as compared to non-induced cells. f) Investigation of the effect of benz(α)pyrene and benz(α)anthracene on DNA synthesized after treatment of WI-38 cells measuring both incorporation and repetitive distribution of label.

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The broader investigation which will be initiated is an attempt to correlate the extent of repair replication with mutational events in mouse L5178Y cells.

4. & 5. Scientific Background and Scope

Our immediate goal is to perform experiments measuring the quantitative and qualitative distribution of repair replication so as to further our knowledge of the repair process, and hopefully to gain insight into the molecular development of cell death, mutation, and carcinogenesis. The phenomena of repair, and earlier studies regarding its distribution in the genome, have been discussed in our initial proposal, and in our first renewal application.

A. Comparative Repair in Normal and Cancerous Cells: Confirmation of Normality

The results of our studies to date, as reported in our second annual progress report (February, 1976: ORO-4761-4), when ultraviolet light is the DNA damaging agent, do not indicate different extents of repair after equivalent exposures in a presumably normal human lymphoblastoid cell line, WIL_2-A3, and a cancerous lymphoblastoid cell line, the Raji line of Burkitt's lymphoma. The comparison of the extent of repair replication occurring after DNA damage in WIL_2-A3 and normal human diploid WI-38 cells, proposed for this third year of study, will serve to confirm the normality of the lymphoblastoid cell system. In agreement with the UV results, the extent of repair replication occurring during MMS treatment was similar in the normal and cancerous cell

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lines. A slightly lower value of incorporated repair replication radioactivity appeared in the DNA of the EB-3 line of Burkitt's lymphoma, as compared to both the WIL_2-A3 and Raji cell lines, after equal UV and MMS treatments. The distribution of the repair replication radioactivity after UV exposure in DNA sequences of different repetitious frequencies was uniform for the normal WIL_2-A3 cells, and for both the Raji and EB-3 lines of Burkitt's lymphoma.

B. Reexamination of the Distribution of Repair Replication Radioactivity Incorporated into DNA During MMS Treatment

In studying the reassociation of repair replicated DNA from WIL_2-A3 cells, our experiments have shown (Progress Report ORO-4761-4) an apparent divergence of the percents of reassociation for repair replicated DNA damaged by MMS at higher C_0t values, as compared to the uniform distribution of semiconservatively synthesized DNA and the non-divergent distribution of UV damaged and repair replicated DNA. It is our intention to confirm this apparent divergence, previously unreported, by performing the kinetic reaction with one DNA preparation over the entire C_0t range.

C. Repetitious Distribution of DNA Repair Replicated at Different Times After Damage

The studies performed to date, and described in our second annual progress report of the repetitive distribution of repair replication radioactivity in the genome after UV or chemical damage, have been based on the repair replication radioactivity incorporated during a long labeling interval post UV

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exposure or during a long MMS damage induction and repair labeling period. The possibility exists that the regions undergoing repair at an early time after the damage are different in repetitious frequency from those undergoing repair at later times post exposure. Based on our current studies of the extent of the repair labeling at different times post UV or MMS treatment of WI-38 cells, we will investigate this question.

D. Age Response

In our initial proposal, we proposed to determine whether the extent of DNA repair after equivalent exposure of cells is the same in individuals of different ages. We are currently growing skin fibroblast cells from individuals of different ages in our laboratory, and will be performing UV and chemical induced damage and repair replication studies to examine this question. We are very much aware that the results can be biased by genetic differences in the individuals of different ages. This will be taken into account in any discussion of detected differences. If significant differences do exist, we would then investigate the repetitious distribution of the repair replication radioactivity.

E. Effect of Metabolic Activation of Polycyclic Hydrocarbons on the Damage of DNA in the Cell and its Repair

In our laboratory, we have previously attempted to induce AHH activity in human lymphoblastoid cells in order to perform these studies. The results were unsuccessful. For other programs, we have investigated the level of AHH in the WIL_2-A3 cells, and were not able to observe detectable levels; this may

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have been the cause of our unsuccessful experiments. Since we have been able to observe detectable levels of AHH in WI-38 cells in culture, and have also been able to induce the enzyme by pretreatment with benz(α)anthracene in vitro, it is our intention to perform experiments which would allow for the induction of the enzymes responsible for the metabolism of benz(α)pyrene through its biohazardous epoxide state. It is our intention to then determine whether the extent of repair occurring in the DNA of induced cells is greater than that occurring upon benz(α)pyrene treatment in non-induced WI-38 cells.

F. Distribution of DNA Synthesis in WI-38 Cells After Treatment With Chemical Carcinogens

These studies, similar to those investigating ^{3}H-thymidine incorporation and its distribution in mouse L-929 fibroblast cell DNA after UV irradiation (as described in our second annual progress report), will be performed upon treating WI-38 cells with the polycyclic hydrocarbons benz(α)anthracene and benz(α)pyrene. Both are known to induce the enzyme aryl hydrocarbon hydroxylase; their effect on normal DNA synthesis in exposed cells, in terms of the repetitious distribution of the DNA synthesized, has not been investigated. This project was initially described in our second year renewal application.

G. Repair and Mutagenesis

Of greatest importance to establishing the significance of repair replication in the cell is an understanding of the relationship of DNA repair to lethality and mutagenesis. We are currently studying the L5178Y thymidine kinase locus

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assay for mutagenesis described by Clive, Flamm, and Patterson (In: Chemical Mutagens. Principles and methods for their detection, vol. 3, edited bv A. Hollaender, Plenum Press, New York-London, pg. 79, 1973). We have been successful in obtaining the mutant clones necessary for these experiments i.e., a BrUdR resistance and thymidine kinaseless cell line. We are currently performing experiments to obtain a revertant cell line with half the thymidine kinase activity of the wild type; it is the revertant which will be employed for the comparison of the extent of repair replication occurring in the total exposed population after a given MMS treatment concentration (and interval), to the extent of forward mutation to the BrUdR resistant cell type, and to the survival of the cells in the treated population.

Figure 1 shows the thymidine kinase activity of wild type and BrUdR resistant cell clones currently being maintained in our laboratory; potential revertants have been isolated in preliminary experiments with ethyl methanesulfonate as the mutagen.

H. Procedures and Experimental Design

All of the procedures and/or experimental designs have been presented in our earlier application and renewal, or described in our two progress reports.

I. Significance

The signfificance of this study, in terms of the environment, biological damage, and repair, has been described in our previous application, renewal, and

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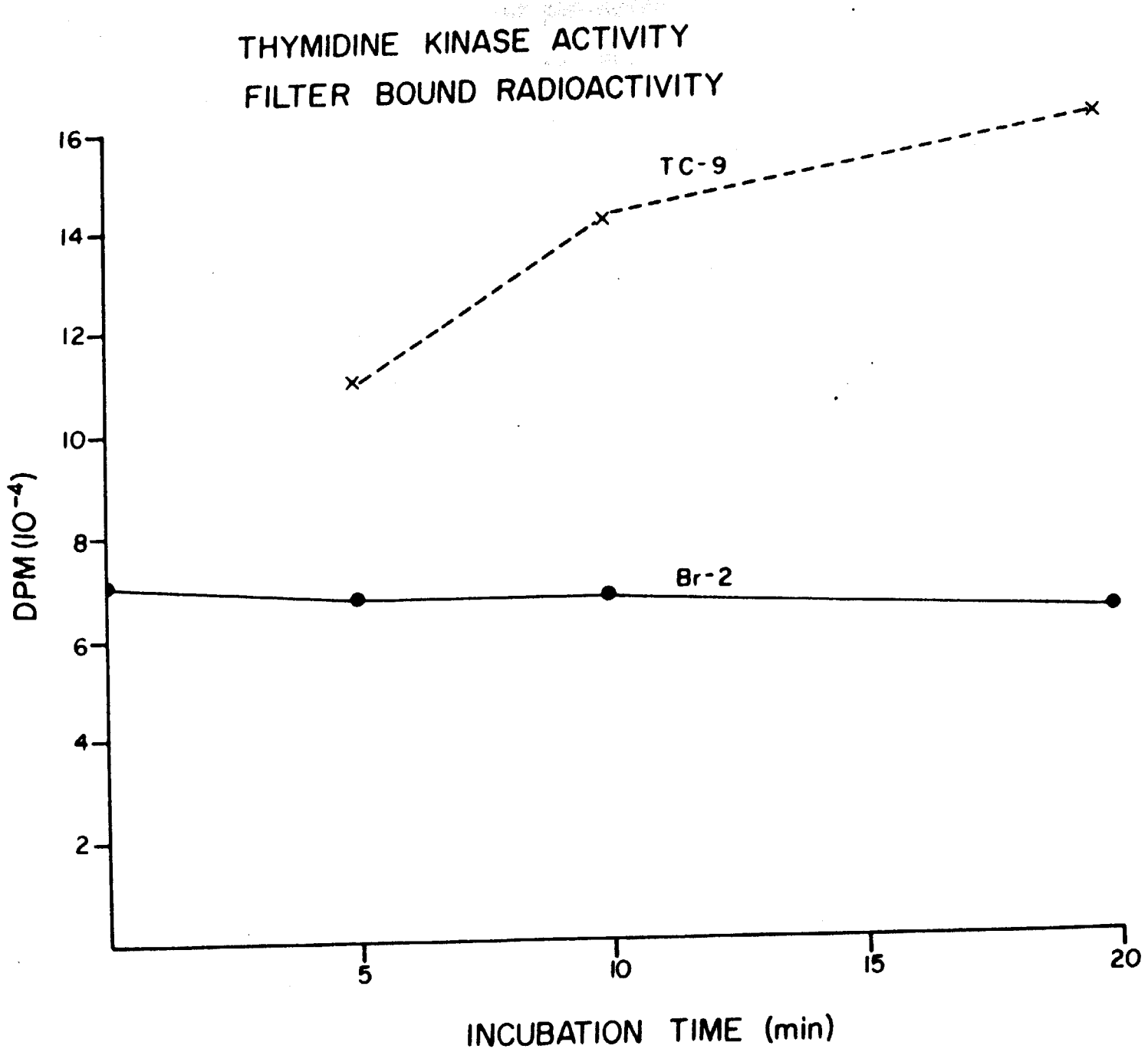
FIGURE 1



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progress reports. Our ability to measure DNA repair makes available to us a tool for accessing the harmful potential at the DNA level of biohazardous agents in the environment.

6. Scientific Personnel

Martin L. Meltz, Ph.D., Principal Investigator

Associate Foundation Scientist

Social Security No.: [redacted]

Percent of time for 12 months: 20%

Date of Birth: [redacted]

Place: New York City, New York

Education:	Degree	Date	Major Field
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]		[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]		[redacted]

Honors:

Atomic Energy Commission Post-doctoral Fellow
National Defense Education Act (NDEA) Pre-doctoral Fellow
Sigma Xi

Major Research Interest:

Cell and molecular biology - actions of chemical and physical agents.

Research Support:

AEC Contract No. AT-(40-1)-4761, "Repetitious nature of repaired DNA in mammalian cells." (June 1, 1974-) Principal Investigator, 25% time.
EPA Grant Project No. R803705, "In vitro assay of relative toxicity of diesel-engine exhausts," (July 7, 1975-July 6, 1977) Principal Investigator, 10% time.
NCI Contract No. N01-CM-67075, "Conduct in vitro cell culture screening of new materials for cytotoxicity," (January 1, 1976-December 31, 1978) Principal Investigator, 20% time.

Research and/or Professional Experience:

September 1971-present	Associate Foundation Scientist, Southwest Foundation for Research and Education, San Antonio, Texas.
September 1973-present	Adjunct Assistant Professor of Radiology, University of Texas Health Science Center at San Antonio, Texas.
October 1970-August 1971	Graduate Research Biophysicist, Laboratory of Radiobiology, University of California, San Francisco.

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Dr. Martin L. Meltz is an Associate Foundation Scientist in the Environmental Science Program of the Southwest Foundation for Research and Education, where he has been employed for 3 1/2 years. He is responsible for the cell and molecular biology aspects of the Environmental Sciences Program.

Dr. Meltz received his [redacted] degree in [redacted] from the [redacted] after attending public school in New York City. He was enrolled in a program in [redacted] before transferring to the [redacted] Dr. Meltz completed his degree in [redacted] His thesis research, under the direction of Dr. Shigefumi Okada, was concerned with the effects of X-irradiation on synthesis of RNA in the mouse lymphoma L5178Y cell line grown in suspension culture. His studies of RNA synthesis as a function of cell cycle time and after X-irradiation required use of pulse labeling, sucrose gradient, and RNA/DNA hybridization techniques.

In October 1969, Dr. Meltz began an Atomic Energy Commission post-doctoral Fellowship under the supervision of Dr. Robert Painter at the Laboratory of Radiobiology, University of California Medical Center in San Francisco, Ca. His studies were involved with the repetitious distribution of the DNA undergoing repair replication after ultraviolet irradiation, and the cell lines studied were the L-929 mouse fibroblast line and the human HeLa 229 cell line. In September 1971, Dr. Meltz arrived at Southwest Foundation for Research and Education and was involved for two years in a research program studying RNA and DNA synthesis in the ventral prostate of rats as a function of age and hormonal status.

In June of 1974, Dr. Meltz was awarded a research contract by the United States Atomic Engergy Commission to study the repetitious nature of repaired DNA in mammalian cells; this program involves a number of different mammalian cell lines and is concerned with chemically-induced damage, as well as ultraviolet light-induced damage. Dr. Meltz spends 25% of his time on this study (AEC Contract No. AT-(40-1)-4761).

Professional Societies:

Radiation Research Society
American Society for Cell Biology
Biophysical Society
Sigma Xi
American Association for the Advancement of Science
Environmental Mutagen Society

Publications:

1. Meltz, M. L. and Okada, S.: Ribonucleic acid synthesis in the cell cycle of L5178Y mouse leukemic cells: Time of replication of the DNA template of rapidly labeled hybridizable RNA. Exp. Cell Res. 67:90-96, 1971.

2. Meltz, M. L. and Okada, S.: Characterization of the rapidly labeled hybridizable RNA synthesized in L5178Y mouse leukemic cells: Hybridization lifetime studies. Biophys. J. 11:582-595, 1971.

3. Meltz, M. L. and Okada, S.: Increased hybridization of RNA synthesized immediately after irradiation in cultured L5178Y mouse leukemic cells. Int. J. Radiat. Biol. 19:287-292, 1971.

4. Meltz, M. L. and Painter, R.: Distribution of repair replication in the HeLa cell genome. Int. J. Radiat. Biol. 23:637-640, 1973.

5. Meltz, M. L.: Nucleic acid precursor incorporation patterns in ventral prostate tissue from rats of different ages. In: Symposium of the Normal and Abnormal Growth of the Prostate, Chapter 20. M. Goland (ed.), Charles C. Thomas, Springfield, 1975.

6. Meltz, M. L.: DNA repair in baboon alveolar macrophages: A system for assessing biohazardous materials. Accepted for publication (August, 1975).

Abstracts:

1. Meltz, M. and Okada, S.: RNA synthesis in lethally irradiated cultured mammalian cells. Radiat. Res. 35, No. 2: Abstract DE-2, 1968.

2. Meltz, M. and Okada, S.: RNA synthesis in L5178Y mouse leukemic cells: RNA/DNA hybridization studies. Biophys. Soc., Abstract TPM-M6, 1970.

3. Meltz, M. and Painter, R.: Non-uniform distribution of repair replication in mouse L-cell DNA after ultraviolet irradiation. Biophys. Soc., Abstract FPM-B5, 1972.

4. Meltz, M., Gause, E. M., and Rowlands, J. R.: Baboon cell systems for environmental hazard determination. I. Alveolar macrophages. Biophys. Soc., Abstract WPM-E5, 1975.

5. Meltz, M. L. and Thornburg, W. H.: Repair replication and its distribution in "normal" and cancerous human lymphoblastoid cell lines after physical and chemical damage. Radiat. Res. Soc., Abstract Be-7, 1975.

6. Meltz, M. L. and Thornburg, W. H.: Repetitious distribution of DNA in mouse L-929 cells synthesized immediately after different ultraviolet light exposures. Am. Soc. Cell Biol., Abstract #559, Nov., 1975.

7. Other Personnel

Ms. Nancy Jo Whittam, B.S., Assistant Research Scientist, is devoting 100% of her time to this project. Mr. Al Arteaga, Laboratory Assistant, spends 50% of his time on this project.

8. Other Financial Assistance

At this time, Dr. Meltz is Principal Investigator on two other projects: EPA Grant Project No. R803705, "In Vitro Assay of Relative Toxicity of Diesel-Engine Exhaust," July 7, 1975-July 6, 1977 (10% time), and NCI Contract No. N01-CM-67075, "Conduct In Vitro Cell Culture Screening of New Materials for Cytotoxicity," January 1, 1976-December 31, 1978 (20% time). Any funds derived from these projects would be used solely for each project, and would not be available for funding any personnel or material for this research program.

9. Premises, Facilities, Equipment, and Materials to be Furnished by the Contractor

All premises, facilities, and equipment will be provided by the Southwest Foundation for Research and Education. The facilities available for this study include a tissue culture laboratory and an adjacent biochemistry laboratory.

a. Tissue Culture Laboratory

The Southwest Foundation for Research and Education has established a Negative Pressure Biohazard Tissue Culture Laboratory for the treatment of mammalian cells in culture with cancer-causing and/or potential anti-cancer compounds. Several programs are currently underway in this and an associated biochemistry laboratory, under the direction of Dr. Meltz, Principal Investigator. These include U.S. E.R.D.A. Contract No. AT-(40-1)-4761, "Repetitious Nature of Repaired DNA in Mammalian Cells," EPA Grant Project No. R803705, "In Vitro Assay of Relative Toxicity of Diesel-Engine Exhausts," and NCI Contract No. N01-CM-67075, "Conduct In Vitro Cell Culture Screening of New Materials for Cytotoxicity."

The Biohazard Laboratory has three separate sterile work rooms and a fourth room in the biohazard area for weighing materials. One of the sterile areas is currently used for non-biohazard work and in it are a Lab ConCo table-top hood, a Model ZBI Coulter counter, a clinical centrifuge, a 37°C air incubator, and a pH meter. The second room is used for biohazard work, and contains a Bioguard vertical laminar flow hood with outside venting and a Forma double-door CO_2 incubator. The third sterile area is a larger biohazard room. This room currently has one vertical laminar flow hood with outside venting, and will shortly have a second vertical laminar flow hood specifically designed for handling carcinogens (with outside venting). Also located in this larger room are a Lab ConCo radiological glove box with a five-place Mettler balance for weighing potentially dangerous chemicals; a UV irradiator, specially designed for our ultraviolet light-induced repair replication experiments; and a full-view air incubator. Immediately adjacent to this larger area is the small room with a four-place balance used for weighing chemicals, and also a 37°C walk-in warm room. The service area of the laboratory has a drying oven and a Wilmot-Castle autoclave. The laboratory is completely separated from the building air conditioning and has its own HEPA filtered air conditioning system, and an airlock at the entrance to the Tissue Culture Laboratory.

Ultraviolet Irradiation Source

An ultraviolet exposure chamber has been built for this work following the specifications of H. Steier and J. E. Cleaver, Laboratory Practice 18:1295 (1969), Exposure chamber for quantitative ultraviolet photobiology.

b. Biochemistry Laboratory

(1) Fume hood

(2) Equipment for DNA isolation

(a) Vortex Genie Mixer
(b) Precision variable control shaker water bath
(c) Eberbach recipricol shaker
(d) International PR-J refrigerated centrifuge

(3) Equipment for shearing DNA

(a) Virtis "65" homogenizer
(b) Aminco DNA cracker

(4) Equipment for hydroxylapatite separation procedure

(a) Batch method

i) Sorvall SS-3 Automatic Centrifuge

(b) Column method

i) ISCO Model UA-5 Absorbance Monitor and optical unit, and ISCO Golden Retriever Fraction Collector.

ii) Water-jacketed column apparatus (as described in progress report: February, 1975)

c. Equipment Available in Southwest Foundation Common Equipment Pool

(1) UV and visible spectrophotometry

(a) Beckman DU Spectrophotometer with Gilford accessories
(b) Cary Model II Recording Spectrophotometer
(c) Spectrofluorimeters

(2) Equipment for scintillation counting

Southwest Foundation for Research and Education has a centralized isotope counting facility. The counting center contains Model 3322 and two Model 3000 Packard Liquid Scintillation Counters which are on line to a G.A. 18/30 Computer, and will also have the unique feature of being under the operational control of the computer as well.

(3) Centrifuge equipment

Beckman Spinco Models L, L2-65, and L3-50 preparative ultracentrifuges are available with a variety of rotors, including two type 50Ti rotors, SW39 and SW25.1 rotors, etc.

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10. Financial Statement

Include in the renewal proposal a financial statement for the present contract period. The statement should be based on the estimated total costs of the project including support by both the institution and ERDA as shown in A-II (a) of Appendix "A" to the Contract covering the current period. The statement should reflect:

a.	Total actual project cost to date for the current period	$79,173
b.	Estimated total cost for remainder of period	$90,000
c.	Total actual and estimated cost chargeable to ERDA for current period based on percentage of cost agreed upon as contained in A-III of Appendix "A" to Contract	$10,827
d.	Accumulated costs chargeable to ERDA (include costs reported in certified statement for preceding period(s) and the costs stated in Item (c) above)	$90,000
e.	Accumulated ERDA Support Ceiling as stated in Article III of Contract	$90,000
f.	Total estimated ERDA funds remaining under Contract (subtract Item (d) from (e)) which may be used to reduce amount of new funds required from ERDA for proposed renewal period	$ -0-

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Budget

	2nd Year Salary	3rd Year Fringe Benefits	2nd Year Total
1. Salaries and Wages	$ 17,020	$2,723	$ 19,743
Scientific Discipline Personnel			
Principal Investigator, M. L. Meltz, Ph.D., Associate Foundation Scientist--20% of time for 12 months Soc. Sec. No. [redacted]	4,800	768	5,568
N. J. Whittam, Assistant Research Scientist--100% of time for 12 months, Soc. Sec. No. [redacted].	9,448	1,511	10,959
Support Personnel			
A. Arteaga, Lab Assistant--50% of time for 12 months Soc. Sec. No. [redacted]	2,772	444	3,216
2. Equipment			$ -0-
None			
3. Travel			$ 250
Domestic			
4. Other Direct Costs			$ 12,300
Supplies and Materials (see attached sheet)			11,000
Publication			300
Communication			100
Computer time, xerox charges, linen, etc.			500
Scientillation Counting			400
5. Indirect Charges - 71.63%			23,131
Total Direct and Indirect Costs .			$ 55,424
Fixed Fee at 6% = $3,325			3,325
Total Project Costs			$ 58,749
Percentage and Amount to be Contributed by Southwest Foundation for Research and Education = 5.66%			$ 3,325
Percentage and Amount Requested of AEC = 94.34%			$ 55,424

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It is the policy of Southwest Foundation for Research and Education that all contracts carry a fixed fee. This is usually an amount equivalent to 6% of the total cost of the contract, which in this case would amount to $3,325. Because of our desire to encourage the undertaking of this research project, which is a component of our Environmental Research Program, we will, as our method of cost sharing, not apply the fee requirement to this renewal proposal.

Justification for Supplies:

a.	Radioactive isotopes, including predominantly ^{3}H-BrUdR, also ^{14}C- and ^{3}H-thymidine.	$ 3,500
b.	Counting vials and scintillation solution chemicals.	2,000
c.	Tissue culture media, serum, disposable tissue culture pipets and labware.	1,500
d.	Cesium chloride and cesium sulfate.	1,500
e.	Chemical reagents.	1,000
f.	Glassware.	1,000
g.	Cost of cell lines from ATCC.	200
h.	CO_2 for Forma incubator.	300
		$11,000

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