UNIVERSITY OF CALIFORNIA, LOS ANGELES
LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY
900 VETERAN AVENUE
LOS ANGELES, CALIFORNIA 90024

ATOMIC ENERGY COMMISSION CONTRACT AT(04-1)GEN-12

RESEARCH AND DEVELOPMENT PROGRAM
FISCAL YEAR 1967

APRIL 1965

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UNIVERSITY OF CALIFORNIA, LOS ANGELES
CONTRACT AT(04-1)-GEN-12

U. S. Atomic Energy Commission
SAN FRANCISCO OPERATIONS OFFICE

Biology and Medicine
PROGRAM

RESEARCH AND DEVELOPMENT PROGRAM

INTRODUCTORY STATEMENT

General Objectives of the Laboratory

The biomedical program of the Laboratory of Nuclear Medicine and Radiation Biology is conducted within the scope of the following categories: Somatic Effects of Radiation; Combating Detrimental Effects of Radiation; Molecular and Cellular Level Studies; Environmental Radiation Studies; Radiological and Health Physics and Instrumentation; Chemical Toxicity; Cancer Research; and Selected Beneficial Applications.

The overall objectives of the Laboratory within these areas of the Biology and Medicine Program may be summarized as follows:

1. Investigation of the effects of ionizing radiation on living organisms and systems of biological significance.

2. Investigation of the dynamic aspects of physiological and biochemical processes in man, animals and plants and how these processes are modified by radiation and related pathological states.

3. The assessment and study of the immediate and long term consequences of the operation or detonation of nuclear devices on the fauna, and flora in man's environment and on man.

4. The development of methods of minimizing or preventing the detrimental effects of ionizing radiation.

5. Research in, and development of, beneficial uses of ionizing radiation and radioactive substances in medicine and biology.

6. Research in the development of new and more efficient radiation detection devices.

7. Research, including field studies, as mutually agreed upon by the Commission and the University, in connection with the conduct of weapon tests and biomedical and civil effects experiments at such tests conducted at continental and overseas test sites.

8. The conduct of training and educational activities in the biological and medical aspects of radiation and related fields.

PROGRAM ASSUMPTIONS FOR FY 1966 and FY 1967:

In general, the overall direction and level of the Laboratory's program remains unchanged during FY 1966, but certain shifts of emphasis within individual projects has resulted in a significant change in the level of effort within three of the categories of the Biology and Medicine Program. The major change occurs in the transfer of all research activity in Atmospheric Radioactivity and Fallout to the category of Terrestrial and Fresh Water Ecology as a result of the shift of emphasis from collection and physical characterization of fallout to studies dealing with the biological cycling of fission products and the development of models to predict the consequences of this cycling. In Radiological and Health Physics a reorientation of the program occurred in FY 1965 with the discontinuance of the work in chemical dosimetry and the initiation of investigations, by optical methods, of the fundamental electronic states of radiation-excited molecules, and the implementation of these new studies is reflected in increased activity in this category.

During FY 1967 moderate expansions are anticipated in some programs, and additional shifts of emphasis in others. In Radiation Effects-General, studies of the clinical aspects of the late effects of irradiation will have been concluded and the planned shift to biochemical investigations of this problem will result in a slight increase in the level of activity. In Molecular and Cellular Level Studies, a moderate expansion is expected to result from increasing emphasis on the effects of ionizing radiation on the function, structure, and ultrastructure of membranes and cells and the initial events associated with the interaction of ionizing radiation and molecules, as well as nucleic and coding systems and related enzyme studies. In Terrestrial and Fresh-Water Ecology there will be an expansion of the studies involving the biological cycling of radionuclides, as well as increased activity in the arthropod studies in connection with the ecological programs at the Nevada Test Site. In Cancer Research a modest increase is planned in the studies of the mechanism of the induction of cancer and leukemia in animals by nucleic acid and virus and its relationship to the induction of cancer and leukemia by radiation. In Selected Beneficial Applications an increase in research activity is expected in the studies dealing with the development of new diagnostic radioisotope procedures and the study of their applicability in medical practice. Emphasis will be placed on organ visualization by various radioisotope scanning procedures, including the development of new test agents and improved scanning equipment. It is anticipated that all other programs will be continued at approximately their current level of effort.

GENERAL COMMENTS ON PROGRAM COSTS:

The composition of costs and staffing for FY 1965, FY 1966, and FY 1967 are summarized below for the entire Biology and Medicine Program by major categories of expense.

	FY 1965	FY 1966	FY 1967
I. Costs: (Shown as Thousands)			
Salaries and Burden (Direct)	$1,333.4	$1,453.1	$1,580.4
Supplies and General Expense	224.6	250.7	270.1
Indirect Costs	1,022.0	1,096.2	1,119.5
Total Operating Costs	$2,580.0	$2,800.0	$2,970.0
II. Manpower: (Shown as Full Time Equivalence)			
Direct Man Years	154 $\frac{1}{4}$	162 $\frac{1}{2}$	171
Direct Scientific Man Years	130 $\frac{3}{4}$	143 $\frac{1}{4}$	150 $\frac{3}{4}$

III. Cost/Manpower Data:

Cost/Direct Man Years	$ 16.7	$ 17.2	$ 17.4
Cost/Direct Scientific Man Years	$ 19.5	$ 19.7	$ 19.7

Total Cost and Manpower data for individual research projects are summarized by Biology and Medicine Activity categories in the chart on Page 4. More detailed cost and manpower data is given in the individual project statements on succeeding pages.

As will be noted in the individual project statements the method used at this Laboratory for allocating indirect costs to research projects consists of prorating total indirect costs on the basis of the percentage of total direct salary expense that each research group incurs. This method of assigning indirect costs is believed to be sufficiently accurate and appropriate for an organization of our size and relative uniformity of composition. However, under this method of proration indirect costs are not specifically identifiable under individual research projects, and for this reason, the composition of indirect expense for the total Program is given below in some detail.

	FY 1965	FY 1966	FY 1967
Composition of Indirect Expense:			
I. Manpower (Shown as Full Time Equivalence):			
Administrative Services	41	41 ½	42
Technical Services	17	17	18
Building Maintenance	8 ½	9	9
Total Indirect Personnel	66 ½	67 ½	69
II. Costs: (Shown in Thousands)			
Administrative Services	$ 381.7	$ 420.3	$ 428.1
Technical Services	165.0	173.9	188.3
Building Maintenance	67.8	79.4	80.5
Occupancy Charge	200.0	200.0	200.0
Utilities	77.5	86.9	86.9
U. C. Management Allowance	75.0	75.0	75.0
U. C. Accounting & Purchasing Services	36.5	36.5	36.5
Miscellaneous (Security, Laundry, Postage, General Supplies, etc.)	18.5	24.2	24.2
Total Indirect Costs	$1,022.0	$1,096.2	$1,119.5

LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY
UNIVERSITY OF CALIFORNIA, LOS ANGELES-CONTRACT AT(04-1)-GEN-12
PROGRAM 060000 COST SUMMARY
(In Thousands)

AEC ACTIVITY / PROJECT TITLE-INVESTIGATOR	FY 1965 COSTS	FY 1965 MAN YRS	FY 1966 COSTS	FY 1966 MAN YRS	FY 1967 COST	FY 1967 MAN YRS
06-01-01 Radiation Effects-General						
Medical Physics Problems (B. Cassen)	$ 52.7	3¼	$ 56.3	3¼	$ 57.9	3¼
Effect of Radiation on the Nervous System (T.J. Haley)	88.3	5½	100.1	5½	102.8	5½
Post-Irradiation Gastrointestinal Function (L.E. Detrick)	54.3	3	53.9	3	56.8	3
Late Effects Radiobiology (L. Bennett and B. Lamson)	28.7	3	33.5	3	54.8	4
ACTIVITY TOTAL	$ 224.0	14¾	$ 243.8	14¾	$ 272.3	15¾
06-03-00 Combating Detrimental Effects of Radiation						
Radiation Therapeutics (J. Leitch - G.V. Taplin)	$ 59.8	3½	$ 55.8	3	$ 59.6	3¼
ACTIVITY TOTAL	$ 59.8	3½	$ 55.8	3	$ 59.6	3¼
06-04-00 Molecular & Cellular Level Studies						
Enzyme Chemistry (I. Harary)	$ 103.6	5	$ 114.5	6	$ 99.1	5
Macromolecular Chemistry (N. Simmons)	34.5	2¾	61.3	3¾	69.9	4¾
General Metabolism (J. F. Mead)	68.1	4¾	69.8	4¾	71.1	4¾
Organic Chemistry (D. R. Howton)	48.7	2¾	55.6	2¾	58.2	2¾
Tracer Synthesis (J. C. Nevenzel)	50.0	2¾	55.2	2¾	57.9	2¾
Pathology (T. G. Hennessy - Acting)	48.0	3½	48.1	3½	49.7	3½
Chemical Radiobiology I (L. S. Myers)	112.5	7	132.5	7	146.5	8
Chemical Radiobiology II (J. F. Ward)	34.5	2	41.0	3	57.7	4
Metabolic Radiobiology (O. A. Schjeide)	105.1	6	122.4	7	124.6	7
Cellular Radiobiology (N. de T. Whittaker)	40.1	4	60.9	4½	89.6	6
Physical Radiobiology (E. H. Strickland)	33.2	2½	59.8	3½	68.2	4½
ACTIVITY TOTAL	$ 678.3	43	$ 821.1	48½	$ 892.5	53
06-05-01 Terrestrial & Freshwater Ecology						
Soil Factors (H. Nishita)	$ 76.7	4¼	$ 64.1	4½	$ 65.9	4½
Plant Factors (E. M. Romney)	76.9	4¼	67.3	4½	82.9	4½
Environmental Decay (H. Hawthorne)	106.1	6	94.8	5½	88.5	5½
Plant Physiology (W. A. Rhoads)	58.8	3¼	58.4	3½	58.9	3½
Radiation Ecology-Mammalian Irradiation (N. French)	110.9	6¼	112.1	6½	114.3	6½
Ecology of the Nevada Test Site (J. Beatley)	85.4	5¼	112.1	6½	115.2	6½
Radiation Ecology-Lizard Irradiation (F. Turner)	103.7	6¼	147.1	8	154.6	8
Radiation Ecology-Plant Irradiation (W. Martin)	74.3	5¼	133.2	7	125.9	7
Chemical Problems-Ecology (R. Wood - Acting)	85.5	5	165.9	10½	187.6	11
ACTIVITY TOTAL	$ 778.3	45¾	$ 955.0	56½	$ 993.8	57

06-05-03	Atmospheric Radioactivity and Fallout						
	Chemical Problems-Fallout Studies (R. Wood - Acting)	$ 74.6	5¾	$ -	-	$ -	-
	Nuclear Events - Biological Studies (F. Turner)	29.7	1½	-	-	-	-
	Environmental Assessments-Plant Studies (W. Martin)	50.5	2½	-	-	-	-
	Physical & Radiological Characteristics of Fallout (K.Larson)	50.4	2¼	-	-	-	-
	ACTIVITY TOTAL	$ 205.2	12	$ -	-	$ -	-
06-06-01	Radiological & Health Physics						
	Excited States of Molecules in Radiation Biology (R.Lehman)	$ 26.1	1¼	$ 48.5	3	$ 51.1	3
	ACTIVITY TOTAL	$ 26.1	1¼	$ 48.5	3	$ 51.1	3
06-06-02	Radiation Instruments						
	Medical Physics Instrumentation (B. Cassen)	$ 69.3	3¼	$ 58.9	3¼	$ 60.5	3¼
	ACTIVITY TOTAL	$ 69.3	3¼	$ 58.9	3¼	$ 60.5	3¼
06-07-00	Chemical Toxicity						
	Chemical Toxicity of Rare Earths (T. J. Haley)	$ 51.2	2½	$ 52.8	2½	$ 53.3	2½
	ACTIVITY TOTAL	$ 51.2	2½	$ 52.8	2½	$ 53.3	2½
06-09-00	Cancer Research						
	Biological Studies of Leukemia (E. F. Hays)	$ 59.8	4¼	$ 61.3	4¼	$ 67.3	4¾
	ACTIVITY TOTAL	$ 59.8	4¼	$ 61.3	4¼	$ 67.3	4¾
06-10-00	Selected Beneficial Applications						
	Clinical Nuclear Medicine (G. V. Taplin)	$ 133.0	7	$ 137.6	7¼	$ 145.9	7
	Basic Nuclear Medicine (G. V. Taplin)	60.2	5	72.6	4½	67.5	4½
	Hemodynamics (G. V. Taplin)	53.2	3	58.5	3	61.0	3
	Nuclide Metabolism (N. MacDonald)	106.3	5½	112.9	6	115.8	6
	Hematology (J. F. Ross)	24.1	½	51.2	3	64.7	4
	Mammalian Radiobiology (T. G.Hennessy)	51.2	3	70.0	3	64.7	4
	ACTIVITY TOTAL	$ 428.0	24	$ 502.8	26¾	$ 519.6	28½
	TOTAL 06-00-00 PROGRAM	$2,580.0	154¼	$2,800.0	162½	$2,970.0	171

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
   University of California, Los Angeles

   Contract No.: AT(04-1)GEN-12

2. Project Title:
   Medical Physics Problems

3. AEC Budget Activity No.:
   06-01-01

4. Date Prepared: April 1965

5. Method of Reporting:
   Publications, UCLA Reports
   Semi-annual and Final Reports

6. Working Location:
   UCLA

7. Person in Charge:
   Benedict Cassen

8. Project Term:
   From: 1963 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2 1/2	2 1/2	2 1/2
(b) Other Technical	3/4	3/4	3/4
Total	3 1/4	3 1/4	3 1/4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 29,600	$ 29,700	$ 30,800
(b) Materials & Services	2,700	4,700	4,700
(c) Indirect Expenses*	(2%) 20,400	(2%) 21,900	(2%) 22,400
Total	$ 52,700	$ 56,300	$ 57,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1964

None (A Ph.D. thesis prepared by Dr. R. Neff entitled "The Effect of Ionizing Radiation on Lymphocytes" was accepted.)

12. SCOPE OF PROJECT

1. Physiology and Effects of Radiation on Lymphocytes

In this project much of our effort is devoted to the use of new techniques and methods in the study of the physiology and effects of acute radiation on circulating lymphocytes. Currently, studies are being made to determine by means of a multichannel analyzer in conjunction with a Coulter orifice the size distribution changes in lymphocyte cell cultures stimulated by phytohemoglutinin and other immunological stimulants. The immunologically stimulated growth of small lymphocytes can be detected at a very early stage long before mitotic figures can be observed. The relative degree of stimulation can be quantitated.

Developments of means of grossly separating viable cells by electrophoresis are being used for the separation of lymphocytes and for the determination of the in vivo and in vitro effects of radiation on the electrophoretic distributions.

A program has been initiated for Dr. R. C. Leif, a post graduate fellow in the Biophysics Department, to continue his researches started at the California Institute of Technology on the use of precision buoyant density distribution methods for grossly separating cell fractions of very small density differences. This method has been applied successfully to circulating erythrocytes. It will be extended to a study of bone marrow cell suspensions with an attempt to concentrate and isolate erythropoetic stem cells.

13. RELATIONSHIP TO OTHER PROJECTS

Dr. M. Ingram, University of Rochester, Atomic Energy Project
Dr. O. Trowell, Medical Research Council, Harwell, Didcot, England
Dr. C. Craddock, UCLA School of Medicine
Dr. E. Cronkite, Brookhaven National Laboratory
Dr. Howard Mel, Donner Laboratory

14. TECHNICAL PROGRESS IN FY 1965

A repeat examination has been carried out by Dr. R. Neff (Ph.D. thesis) on the effects of in vivo and in vitro irradiation on the size distribution of rabbit lymphocytes. Contrary to his earlier measurements with a single channel analyzer, he found that for both in vivo and in vitro situations the relative size distributions were unchanged although the numbers in both cases were greatly reduced. He also found, on the basis of two modes of calibration, that there were practically no lymphocytes with volumes of corresponding diameters over 9 microns. This surprising result can only be explained on the basis of the fact that in the usual methods of characterizing large lymphocytes the larger cells are considerably flattened and squashed out to a larger apparent diameter. Comparisons were made of smeared cells on a slide and cells dropped on a slide, and larger diameters were found on the smears than on the slides with the cells dropped on.

1. By the use of the logarithmic display feature of the multichannel analyzer, the new linear amplifier, and an improved Coulter aperture, it has been found easy to detect the increase in size of small lymphocytes subject to photohemoglutinin or cross immunological stimulation. The lymphocytes are grown in a cell culture. Their stimulated increase in size can be observed long before mitotic figures can be observed after the use of colchicine. This method is being evolved to quantitate the relative degree of immunological stimulation.

2. In the course of the development and testing of improved electrophoretic cell separations further observations have been made of the fact that viable cells can be fractionated electrophoretically. The effect of temperature on the viable electrophoretic zeta potentials is being studied.

3. Equipment has been set up to enable precision buoyant density gradient fractionation of cell suspensions. First tests are being made to grossly separate old erythrocytes which are the densest and make measurements of their biophysical properties. The equipment is designed and is being built to explore the possibility of concentrating and separating erythropoetic stem cells from bone marrow suspensions. Preparations are under way to use the stem cell assay method of Till.

15. EXPECTED RESULTS IN FY 1966

It is expected that there will be a continuation of thorough and systematic measurements of changes in lymphocyte size distributions after in vivo total body irradiation through the complete sequence of injury, rebound and recovery. It is expected that similar measurements will continue on electrophoretic fractions of these cells. Experiments will be initiated to determine whether or not the survivors of irradiation show the same immunological stimulation responses as the unirradiated cells.

It is expected that buoyant density methods can be used to separate and study the biophysical properties of red cells near the end of their life. It is expected that erythropoetic stem cells can be concentrated and separated.

16. EXPECTED RESULTS IN FY 1967

It is anticipated that in FY 1967 there will be a continuation of the development of techniques and methods for measuring properties of lymphocytes and that these will be applied to the study of the complex physiology and radiation biology of these cells. It is expected that a program will be initiated to determine the effects in vitro and in vivo of various agents such as certain pharmacologically active agents, polypetides, etc., on cell size distribution and electrophoretic mobility. It is expected that cell size distribution and electrophoretic mobility changes can be observed in animals and in human blood in various disease states.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Effect of Radiation on the Nervous System

3. AEC Budget Activity No.:
06-01-01

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Thomas J. Haley

8. Project Term:
From: 1955 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 ½	4 ½	4 ½
(b) Other Technical	1	1	1
Total	5 ½	5 ½	5 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 43,000	$ 50,600	$ 53,900
(b) Materials & Services	4,400	5,700	4,200
(c) Indirect Expenses*	(4%) 40,900	(4%) 43,800	(4%) 44,700
Total	$ 88,300	$100,100	$102,800

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Plasma and Adrenal Gland Corticosterone Levels Following X-Ray Exposure in Rats, Hameed, J.M. Abdul and Haley, Thomas J., Radiation Research, 23, 620-629 (1964).

2. Influence of Physostigmine and Neostigmine on the Responses of Goldfish Intestine to Acetylcholine, Haley, Thomas J., Colvin, G. and M. Efros, Jour. of Pharmaceut. Sciences 53, 1530 (1964).

3. A Cast Electrode Mount for Self-Stimulation Electrodes, Haley, T.J., Flesher, A.M., Flygare, K. and R. Myers, Jour. of Pharmaceut. Sciences, 54, 164 (1965).

4. Quinoxaline-di-N-Oxide, Radioprotector and Radiosensitizer, Haley, T.J., Trumbull, W.E., and Cannon, Jack A., Advances in Biochemical Pharmacology, Vol. I., Radiosensitizers and Radioprotective Drugs, Basel, S. Krager, in press.

5. Reserpine, Tetrabenazine and Iproniazid Effects on Self-Stimulation, Haley, T.J. and Flesher, A.M., Proc. XXIII Intl. Cong. of Physiological Sciences, Tokyo, Sept. 1965, in press.

6. Psycho-Sedative Properties of Some (Indolylethyl) Piperazine Derivatives, Hameed, J.M. Abdul and Haley, T.J., Proc. West. Pharmacol. Soc., in press.

7. Blocking UDP Spasmogenic Action with Papaverine Derivatives, Haley, T.J. and Christensen, D., Fed. Proc., in press.

8. Response of the Nervous System to Ionizing Radiation, 2nd Intl. Symposium T.J. Haley and Ray S. Snider, Little Brown and Co., Boston, 1964.

12. SCOPE OF THE PROJECT

Although information is available on certain aspects of the physiology of the irradiated animal, further studies are necessary if we are to understand and counteract the physiological changes produced by exposure to ionizing radiation. Such studies should include an estimation of particular physiological responses and their correlation with observed cytological effects. This is particularly true for the presumed radioresistant tissues, e.g. nervous system, which do not present gross evidence of cytological damage, but may be incapable of performing their required physiological function in the normal manner. Of equal importance is the study of the return of normal functional capacity following both acute and chronic exposure to ionizing radiations. Quantitative estimation of tissue or organ secretions e.g. acetylcholine, epinephrine, norepinephrine, etc. will also be of assistance in determining the functional capacity of such systems and any significant alteration in precursos-hormone content will be indicative of physiological changes resulting from radiation exposure.

13. RELATIONSHIP TO OTHER PROJECTS

U.S. Air Force Aviation Medical Lab.; U.S. Dept. of Defense; Armed Forces Institute of Pathology; Baltimore City Hospital; Columbia University; University of California, Berkeley, Los Angeles; University of Chicago; Cornell University; Florida State University; University of Georgia; Institute for Cancer Research; Institute of Living; Johns Hopkins University; Massachusetts Eye and Ear Infirmary; Massachusetts General Hospital;

Methodist Hospital; University of Michigan; University of Minnesota; University of Nebraska; New England Deaconess Hospital; Northwestern University Medical School; State University of New York; University of Notre Dame; University of Oregon; C.W. Shilling Auditory Research Center, Inc.; Texas Technological College; Texas Medical Center; Texas State College; Vanderbilt University; Medical College of Virginia; Washington Hospital Center; Wayne State University; Worcester Foundation for Experimental Biology; Yerkes Laboratories.

14. TECHNICAL PROGRESS IN 1965

Determinations of blood and adrenal gland corticosteroids show two peaks in activity, one at 2.5 hours and the other 48 to 72 hours after irradiation. The steroid concentrations in the adrenal gland remained elevated for 48 hours, decreased on postirradiation days 5 and 7, and returned to control levels by day 14. Irradiation of the head alone produced a rise in both plasma and adrenal gland steroid levels at 72 hours with return to control values on the succeeding day. Irradiation of the body only resulted in an increase in the steroid concentrations of the plasma and the adrenal gland at 2.5 hours, with a return to control levels by 24 hours. A method has been developed using the isolated goldfish intestine which accurately determines acetylcholine at 10^{-9} grams. Also, present work indicates that the uridine phosphates which interfere with this assay can be selectively blocked with some synthetic papaverine derivatives. Successful spectrophotofluorometric analyses are now being made in adrenal corticoids, serotonin and catechol amines, thus allowing a comparison to be made of the brain content of these materials in radiated and non-radiated bar pressing rats. It is hoped correlations can be obtained between the level of brain activity and the presence of these materials.

15. EXPECTED RESULTS IN FY 1966

Work will continue on quantitative estimation of brain neurohormones under the influence of both radiation and drugs. Preparation of Substance P and the correlation of its presence in the nervous system with X-ray induced electrical changes will be undertaken.

16. EXPECTED RESULTS IN FY 1967

In cooperation with UTAEC Agricultural Experiment Station, analyses will be undertaken of the corticoid content and the mineral elements of the blood of burros subjected to neutron irradiation. The functional capacity of selected structures in the autonomic nervous system will be studied in an effort to correlate the conduction of the nervous impulse with neurohormone secretion during acute irradiation injury and recovery. Both biological and chemical analysis will be made of neurohormone content of the irradiated brain to ascertain what changes, if any, occur under the influence of ionizing radiation and what their relationship to brain electrical activity may be. Studies will also be initiated applying EEG analysis plus psychological approach techniques to the study of radiation induced nervous system changes.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Post-Irradiation Gastrointestinal Function

3. AEC Budget Activity No.:
06-01-01

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Lawrence E. Detrick

8. Project Term:
From: 1948 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3	3	3
(b) Other Technical	0	0	0
Total	3	3	3

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 31,100	$ 29,700	$ 31,900
(b) Materials & Services	2,800	2,300	2,500
(c) Indirect Expenses*	(2%) 20,400	(2%) 21,900	(2%) 22,400
Total	$ 54,300	$ 53,900	$ 56,800

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

At present, information on gastrointestinal function of the irradiated animal is restricted to the early phase of injury and little is known concerning the nutritional status of the organism and the contribution made by this factor to the irradiation syndrome. It is essential that more information be obtained on the total sequence of events occurring in irradiation injury and recovery of physiological functions of the gastrointestinal tract. Post irradiation studies on gastrointestinal absorption of essential metabolites (vitamins, amino acids, etc.) may give an insight into the manner in which tissue repair and regeneration may be accelerated and indicate those substances which must be given parenterally in order to increase the rapidity of such processes. Such studies should also indicate the degree of impairment of absorption, the days during which it occurs and the time required for a return to normal physiological function as contrasted with cytological evidence of repair. In addition it will be necessary to investigate possible defects produced in the metabolic conversion pathways in the liver, because homeostasis cannot be maintained even with adequate nutrition, if the organism is unable to transform and utilize metabolites in the repair process.

13. RELATIONSHIP TO OTHER PROJECTS

Argonne National Laboratory; Department of Defense, Armed Forces Institute of Pathology; Brookhaven National Laboratory; University of California, Los Angeles; The University of Cincinnati; Cornell University Medical College; Harvard University; Massachusetts General Hospital; National Research Council Committee on Shock; University of Texas; Vanderbilt University.

14. TECHNICAL PROGRESS IN FY 1965

Work has continued on the following problems: Histological changes from beta-irradiation by gold plated Sr^{90} beads implanted in the rat femur; postirradiation intestinal lipid absorption ultrastructure; and post-irradiation intestinal absorption of tryptophane.

15. EXPECTED RESULTS IN FY 1966

PR-intestinal absorption of L'Methionine. PR-intestinal lipid absorption ultrastructure employing a constant PR-time and a split dose of lipid, and a variable time after abdominal irradiation and a single lipid dose. Switch from totalbody to abdominal irradiation and the determination of survival following single exposures from 800 r to 1500 r; survival after 6 weekly 1000 irradiations. Intestinal absorption of the latter follow an 8 week recovery period and a second irradiation challenge.

16. EXPECTED RESULTS IN FY 1967

A new Siemens electronmicroscope will be employed to investigate post-irradiation intestinal lipid absorption ultrastructure immediately following, and intestinal epithelial recovery from a second exposure after a six week interim, in animals that simulated the clinical X-ray course used to treat gastrointestinal cancer. Radiotagging and/or autoradioautography are procedures being considered for determining changes in postirradiation lipid absorption characteristics.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Late Effects Radiobiology

3. AEC Budget Activity No.:
06-01-01

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Leslie R. Bennett

8. Project Term:
From: 1950 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	2	3
(b) Other Technical	1	1	1
Total	3	3	4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 15,100	$ 18,700	$ 28,600
(b) Materials & Services	3,400	3,800	3,800
(c) Indirect Expenses*	(1%) 10,200	(1%) 11,000	(2%) 22,400
Total	$ 28,700	$ 33,500	$ 54,800

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

The purposes of the research of this section are to study the diseases and longevity observed in previously irradiated animals, and to compare this information with the spontaneous disease pattern and natural longevity of the species. Many variables such as age, sex, total irradiation dose, fractionation and spacing of irradiation dose, and portion of the body irradiated may possibly influence longevity and the type of pathology observed. Studies are required to systematically assess the influence of these various factors in order to more properly define the conditions of exposure to ionizing radiation that present a long term hazard. In addition, knowledge from such studies will help define the pattern of altered physiology in the totally irradiated animal which must be explained by any comprehensive theory or model of delayed radiation injury. Basic information related to the natural aging process can also be expected.

13. RELATIONSHIP TO OTHER PROJECTS

No other project in this Laboratory is primarily concerned with longevity and the delayed somatic effect of total and partial body radiation. A large number of investigators have been, or are now, concerned with the delayed effects of irradiation, among them the following: Storer (Bar Harbor); Mole (Harwell); Howland, Hursh, Casarett (Rochester); Anderson (UC-D); Kaplan (Stanford); Conard, Bond (Brookhaven); Upton (Oak Ridge); Gowen (Univ. of Iowa); and Bustad (Hanford).

14. TECHNICAL PROGRESS IN FY 1965

The chronic NaCl toxicity test for latent irradiation injury in the rat was used to evaluate the possibility of kidney damage from Hg^{203} administered in the clinical dose range. The study is only partially completed. However, preliminary analysis of the results indicate that conventional doses, in the range of 5 to 10 μc per Kg, of Hg^{203}, produce measurable injury.

The studies of chromosome aberrations have shown a rapid clearance of these abnormal cells from rat bone marrow and kidney. The low level of grossly abnormal cells after 30 days would appear to limit their value as a measure of latent radiation injury and these studies have been discontinued.

15. EXPECTED RESULTS IN FY 1966

The toxicity study with Hg^{203} will be completed and reported. Carbon tetrachloride and other forms of biochemical stress will be studied as a means of bringing out latent injury.

16. EXPECTED RESULTS IN FY 1967

While work will continue to revolve around the basic problem of the nature of life shortening, it is apparent that the largely descriptive clinical aspects of this problem are completed. On the other hand, the changes at the biochemical level have been described only in a very superficial manner.

With the rapid growth of knowledge in the fields of nucleic acid and protein synthesis, and in the control of energy processes we are rapidly acquiring the methods and background needed to make a biochemical approach to the late effects of irradiation. The very successful investigations of Chance in the study of glycolysis and energy metabolism by combining computer simulation and laboratory testing suggest the possibility of identifying critical metabolic events in radiation injury and the aging process by application of these methods. During recent years we have been gaining experience with this approach in a study of electrolytes (Bradham et al*). Preliminary explorations will be made into both the mathematical models and the laboratory measurement of potentially significant reactions.

*Isotope Dilution and Thermodynamics in the Study of Intercompartmental Body Fluid Exchange. Bradham, Bennett, Dehaven, Deland, Wolf, and Maloney. Surg. Gyn. and Obst. 119, 1062-68, (1964).

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Radiation Therapeutics

3. AEC Budget Activity No.:
06-03-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
James L. Leitch (deceased)
George V. Taplin, M.D. (acting)

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3 ½	3	3 ½
(b) Other Technical	0	0	0
Total	3 ½	3	3 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 34,000	$ 27,500	$ 30,800
(b) Materials & Services	5,400	6,400	6,400
(c) Indirect Expenses* (2%)	20,400 (2%)	21,900 (2%)	22,400
Total	$59,800	$55,800	$59,600

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

The following publications were prepared by Dr. J.L. Leitch (deceased) and will be published in FY 1965.

Leitch, J.L.: Comparison of protection against X- and γ-radiation by AET plus 5-HT. (to be submitted to Health Physics).

Leitch, J.L.: X-irradiation protection studies III. Total dose and rate effects in x-ray protection studies. (to be submitted to Health Physics)

Leitch, J.L.: Chemical protection in health physics. (to be submitted to Scientific American).

Leitch, J.L.: Radiation protection studies. AET in DMSO as an effective radiation protection agent. Preliminary Report (to be made into UCLA report).

12. SCOPE OF THE PROJECT

Objectives are to study the effects of whole body irradiation and to devise therapeutic means of combating the detrimental effects of ionizing radiation. This work includes the investigation of the dose-rate-therapy relationships for various drugs, the development of new therapeutic agents, modification of old compounds or combinations of both old and new, the development of special agents for localization in specific tissues or groups of tissues, and also to evaluate the differences, if any, between x-irradiation and gamma-irradiation as related to treatment. In addition, effects of ionizing radiation will be evaluated in terms of possible radiation mechanisms as elucidated by pharmacological means.

13. RELATIONSHIP TO OTHER PROJECTS

Oak Ridge Institute of Nuclear Studies; Brookhaven National Laboratory; Argonne National Laboratory; Los Alamos Scientific Laboratory; Naval Radiological Defense Laboratory; Western Reserve University and various research centers in numerous foreign countries including Sweden, Japan, Germany, England, Italy and Belgium.

14. TECHNICAL PROGRESS IN FY 1965

Current interest in dimethylsulfoxide (DMSO) and indications that it might have some protective value against whole-body irradiation damage led to studies of the compound alone and in combination with AET with promising results.

Six groups (60 mice each) were exposed to a total dose of 1091 r whole body Co^{60} gamma irradiation delivered at 242 r/min. Two groups received no pretreatment. The other four were given a standard I.P. injection of 0.20 ml per mouse of 90%, 80%, 70% and 60% DMSO, respectively. All pretreated mice showed a marked increase in mean survival time of from 397 to 252% indicating that DMSO alone showed definite protection under the experimental conditions.

The effect of adding AET was demonstrated as follows: One solution contained 20 μ M AET in the standard volume of 0.20 ml prepared in water; a second contained the same amount of AET but in 75% DMSO. Mice were intended to be exposed to approximately 1010 r at 3 different radiation dose rates (235, 68.6 and 14.7 r/min) but an error in distance between source and exposure box gave a higher total dose (1187 r) for the 68.6 r/min experiment. Results showed that whereas AET in aqueous solution has a pronounced decrease in protection

with decreasing radiation rate (= longer exposure time), no such decrease is observed when the AET is prepared in 75% DMSO, and the latter solution apparently affords approximately the same protection at all three radiation rates.

Life Span Studies: (Still incomplete) indicate that treated mice which survived the initial effects of radiation, have a life span approximately two-thirds that of the non-irradiated controls.

Post-Irradiation Uterine Response to 5-HT: To obtain further data on treated mice following lethal doses of X- and γ-radiation studies of the contractile responses of the superfused isolated mouse uterine horn to 5-HT have been initiated, using the bioassay procedure previously described (Leitch et al 1957). Preliminary results indicate reduced sensitivity of the uterine horns of irradiated animals as compared to non-irradiated controls. (Uterine horns and ovaries of irradiated animals exhibit smaller dimensions than those of the non-irradiated controls).

High Intensity γ-Irradiation Studies: Exposure of mice at the center of the 10,000 curie Co^{60} source at a dose rate of approximately 100,000 r/min show animals dead when removed from source if exposure is 32 sec. or longer. After exposure of 1,2,4 or 8 seconds mice survive 3-5 days with little or no difference in survival time. Pretreatment with 10 μ M AET + 1 μ M 5-HT showed only slight protection. Plans to monitor CO_2 output of mice while in the center of the source by means of a Beckman infrared analyzer are underway. Preliminary studies on CO_2 output of mice after irradiation show that such animals exhibit a reduction in CO_2 even in those cases where nervous system damage has produced activity ("Jumpiness")

15. EXPECTED RESULTS IN FY 1966

Emphasis will be placed on further investigation of the protective effects of DMSO alone and in conjunction with AET and 5-HT in mice following whole body exposures to X- and γ-radiation. These studies will include determination of the duration of action of DMSO after intraperitoneal injection and various other routes. Attempts will be made to prolong DMSO action by combining it with other materials to release the DMSO more slowly from the sites of injection and to prepare colloidal suspensions of DMSO to localize it in the liver, spleen and bone marrow. Its radiation protective effects and toxicity will also be studied in other animal species.

Because of the reported value of DMSO in thermal injury (freezing and/or burning) its healing effects on radiation burns will be studied using local applications made before and after radiation exposures to X-, γ-and beta radiation sources. Should the DMSO have protective action against radiation injury of the skin, its use in radiation therapy for skin cancer should be beneficial. The fate and organ distribution of DMSO will be studied using DMSO labeled with sulphur-35 or possibly with selenium-75 if this can be accomplished. With the latter label, organ distribution and the routes of excretion of DMSO can be determined quickly but qualitatively by whole body scanning, because the Se^{75} emits an energetic gamma ray. More complete investigation of DMSO metabolism can then be made by serial sacrifice, organ assay and autoradiography using the Se^{35} labeled compound.

16. EXPECTED RESULTS IN FY 1967

It is likely that only part of the investigations listed for the prior year will be completed. Therefore similar studies will be continued and modified according to the results of the prior year's work. It is hoped that it will be possible to incorporate DMSO into colloidal albumin or other protein

particles because of its unique property of penetrability through membranes and bacterial cells. If the action of DMSO can be prolonged by employing it in colloidal form or in depot preparations, its radiation protection action will be investigated in animals exposed at low dose rates to simulate the situation in a fallout area in the event of nuclear warfare. Emphasis will also be placed on exploiting its radiation protection effects in radiation therapy for cancer and leukemia.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
Enzyme Chemistry

3. **AEC Budget Activity No.:**
06-04-00

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
Issac Harary

8. **Project Term:**
From: 1960 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 ½	5 ½	4 ½
(b) Other Technical	½	½	½
Total	5	6	5

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 47,400	$ 55,100	$ 49,600
(b) Materials & Services	13,100	13,400	13,500
(c) Indirect Expenses*	(4%) 43,100	(4%) 46,000	(3%) 36,000
Total	$ 103,600	$ 114,500	$ 99,100

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Kuramitsu, H. and I. Harary, Studies In Vitro on Single Beating Rat-Heart Cells. III. Enzyme Changes and Loss of Specific Function in Culture, Biochim. Biophys. Acta, 86 (1964) 65-73.

2. Fujimoto, A. and I. Harary, Studies In Vitro on Single Beating Rat-Heart Cells. IV. The Shift from Fat to Carbohydrate Metabolism in Culture, Biochim. Biophys. Acta, 86 (1964) 74-80.

3. Harary, I. and E. Sato, Studies In Vitro on Single Beating Heart Cells. V. Changes in Adensoine-Triphosphate-Induced Contractions of Extracted Models, Biochim. Biophys. Acta, 82 (1964) 614-616.

4. Harary, I., A. Fujimoto, and H. Kuramitsu, Enzyme Changes in Cultured Heart Cells, National Cancer Institute Monograph No. 13.

5. Harary, I., Studies on Individual Heart Cells, Supplement II to Circulation Research, Vols. XIV and XV, November 1964.

6. Cedergren, B. and I. Harary, In Vitro Studies on Single Beating Rat Heart Cells. VI. Electron Microscopic Studies on Single Cells, J. Ultrastructure Res., 11, 428 (1964).

7. Cedergren, B. and I. Harary, In Vitro Studies on Single Beating Rat Heart Cells. VII. Ultrastructure of the Beating Cell Layer, J. Ultrastructure Res., 11, 443 (1964).

8. Harary, I. and E.S. Slater, In Vitro Studies on Single Beating Rat Heart Cells. VIII. Effect of Metabolic Inhibitors, Biochim. Biophys. Acta, In Press.

12. SCOPE OF THE PROJECT

We are continuing our study of the relation of specific metabolism to specific function using the cultured heart cells as a model. Our project is designed to investigate the control of metabolism as it is designed to bring about specific function and synthesis of specific proteins. As markers of function we use the cells ability to beat, the synthesis of muscle proteins, such as myosin, and the visible sub-cellular structure such as the mitochondria and myofibrils. We are attempting to correlate these measures of function with levels of certain key enzymes of glycolysis, tricarboxylic acid cycle, and fatty acid oxidation. We seek to determine the control of metabolic function and nutrition and the relation of these parameters to specific function in the intact mammalian cell.

Information of this sort will help us understand how basic information from the gene is utilized to determine the function of the cell. Thus radiation effects on mammalian cells will be more adequately pinpointed and explained.

13. RELATIONSHIP TO OTHER PROJECTS

The following workers in other laboratories are investigating similar problems:

H. Green, The Synthesis of Cartilage Precursors by Cells in Culture; E. Goldwasser, The Effect of Erythropoietin in inducing hemoglobin synthesis in erythroblasts in Culture; Rabinowitz,

The Synthesis of Myosin in Sub-cellular Heart Fractions; and Evans et al., Relation of Lipids to Heart Function.

14. TECHNICAL PROGRESS IN FY 1965

The purpose of our study of single heart cells in culture is to determine the relation of specific metabolism to specific function.

The close relation of lipid metabolism to function is demonstrated by the fact that cells grown in media free of lipids do not beat. Cells grown in a medium supplemented with lipids beat for a longer period.

We now find that addition of lipids, to cells which have stopped beating due to lipid deficiency, will reinitiate beating. This phenomenon occurred also with the addition of fatty acids such as palmitate and to a smaller extent by addition of octanoate and acetate. All of these act after a lag of 2 to 3 hours. Lecithin, on the other hand, acts more quickly after a lag of only 30 minutes.

The effect of lipids could be due to:

1. The oxidation of fatty acids which serves as a specific source of energy for beating.

2. The role of lipids in membrane function.

3. The metabolic stimulus of lipids for specific protein synthesis.

To investigate these possibilities we have initiated three related programs with the following results:

By the use of metabolic inhibitors it has become apparent that beating heart cells will utilize, for the beating function, ATP from any source, such as glycolysis, oxidative phosphorylation, succinic thiokinase or external additions. This would seem to rule out possibility 1.

We are now studying the relation of lipids to membrane function in the heart cells. Whole cell membrane function is being studied by amino acid permeability. Mitochondrial membrane function is being investigated by studying respiratory control, effect of ATP on swelling, and DPNH oxidation.

Myosin synthesis in the intact cells and in sub-cellular fractions is under investigation. We have established a system where myosin synthesis and the parameters which effect its rate and amount can be studied.

We find that the presence of lipids also affects enzyme levels in the intact cell. This indicates that lipids have some relationship to enzyme synthesis.

15. EXPECTED RESULTS IN FY 1966

We will continue to study the relation of lipids to heart function. We expect to relate membrane function to lipids and try to correlate this with heart cell function. We also expect to establish a myosin synthesis system so that such things as the relation of membrane function, lipid metabolism and fatty acid oxidation to myosin synthesis may be studied. We also intend to follow up the observations that lipids affect the enzyme level. It may be that the lipid effect is a direct result of an affect on protein synthesis or that it is related to enzyme synthesis

indirectly by its affect on membrane function.

16. EXPECTED RESULTS IN FY 1967

We want to look more closely at protein synthesis in the heart cells. We plan to study the physical and chemical parameters that affect the quantitative and qualitative aspects of protein synthesis. We want to know more about the mechanism of the effect of lipids. It is our feeling that lipids may in someway control the expression of the genetic material for the function of the heart cell.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Macromolecular Chemistry

3. AEC Budget Activity No.:
06-04-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Norman S. Simmons

8. Project Term:
From: 1950 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	3	4
(b) Other Technical	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{4}$
Total	$2\frac{3}{4}$	$3\frac{3}{4}$	$4\frac{3}{4}$

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 17,300	$ 28,300	$ 36,000
(b) Materials & Services	5,000	8,900	9,300
(c) Indirect Expenses*	(1%) 12,200	(2%) 24,100	(2%) 24,600
Total	$ 34,500	$ 61,300	$ 69,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

Investigations into the relationship between structure and function in biological high polymers, such as the nucleic acids and proteins (structural, enzymic and hormonal) as well as small cytoplasmic particles and viruses, are rapidly assuming a most important role in biological research. The biological function of most macromolecules is largely dependent upon the specific steric relationship of active sites within or upon their surface. Minor changes in the gross configuration of such molecules frequently lead to the diminution or destruction of their biological activity. Further, the biological properties of small cytoplasmic particles and viruses are not only dependent upon the structural integrity of the individual molecular species of which they are constructed, but also to a large degree upon the steric relationship of the different molecular species within their grosser structure (nucleoproteins, liproproteins and glycoproteins).

It is the purpose of this Section to study and characterize the macromolecular configuration of the nucleic acids, proteins, viruses, etc., by all the physical means at our disposal. These include sedimentation, diffusion, viscosity, flow birefringence, light scattering, X-ray diffraction, ultraviolet and infra-red spectrophotometry, rotatory dispersion, etc. It is hoped that these investigations will contribute further to the understanding of the relationships existing between structure and biological activity.

This is a necessary prerequisite to the investigation and understanding of the denaturation, degradation or other manifestations of damage by environmental changes or agents such as ionizing radiation.

13. RELATIONSHIP TO OTHER PROJECTS

Related projects supported by AEC funds are too numerous to mention here. However, major programs in this area are also being conducted at Harvard (Doty, Blout), Cornell (Scheraga), Duke (Tanford) and Univ. of Calif. at Berkeley (Tinoco, etc.).

14. TECHNICAL PROGRESS IN FY 1965

In order to be able to study the metabolism of nucleo-histones in leukemic mice a micro electrophoretic technique has been developed. This will permit the complete electrophoretic analysis of mouse serum on a single drop of whole blood obtained from the tail vein. The obvious advantages are numerous, but, in particular this will permit following the changes in serum protein distribution in mice developing leukemia and then most importantly, it will permit serial studies on the same mouse following irradiation. A description of this technique is in the process of being prepared for publication.

The problem of instrumentation for the conformational analysis of proteins, nucleic acids, and viruses has been actively pursued by the purchase of a Bendix Spectropolarimeter. This excellent instrument has been further improved by the addition of an external AC-DC well regulated power supply capable of operating lamps up to 1000 W, with the appropriate igniter. The cooperation of various lamp manufacturers has been obtained in the development of new lamps with high outputs of ultra-violet light so

necessary to the successful penetration of the important absorption hands of proteins and nucleic acids. Further improvements are being constantly made in the optical system and the photomultiplier systems. Already new conformational dependent cotton effects have been found in proteins.

Further, it has been found, for the first time, and made possible only by improved instrumentation, that the bases of the nucleic acid in the intact Tobacco Mosaic virus are capable of being randomized or disoriented by suitable solvent adjustments. These results are now being prepared for early publication.

15. EXPECTED RESULTS IN FY 1966

Due to the development of superior micro-electrophoretic techniques for measuring serum proteins in single drops of mouse blood, a study of the metabolism of nucleohistones will be undertaken. It is proposed to transplant viable leukemic tissue into adult AKR mice and follow the serum protein profile, with time, as the tumor develops. When sufficient massive nodes are developed the mice will be irradiated and the serum proteins studied with the purpose of detecting the presence of circulating nucleohistones, or their products, following cellular disintegration and disorption of the nuclei of the tumor mass. It is hoped that sufficient evidence will be found to apply other useful techniques of protein analysis to this problem. This, we expect, may have important implications in the pathogenesis not only of the acute post-irradiation syndrome but of all acute and chronic degenerative processes, based upon serum protein interactions with basic histones or by-products such as lysine or arginine rich basic polypeptides.

The conformational analysis of nucleic acids, proteins and viruses will continue to be studied by optical rotatory dispersion techniques with the purpose of detecting not only gross conformational dependent cotton effects, but also of smaller but more important effects due to side chain interactions of aromatic amino acids. This will have important implications in the relationship of structure to function at the active sites of a variety of enzymes.

16. EXPECTED RESULTS IN FY 1967

The following year we will explore this further, as necessary, so as to attempt to formulate a model system for the pathogenesis of the acute post-irradiation syndrome, based upon this early model of protein interactions. In the event that this concept should prove irrelevant or inconsequential, it will be abandoned in favor of pursuing such evidence as might illuminate the problem from other directions. Whatever the outcome it will be pursued vigorously and with real interest.

It is expected that the conformational analysis of macromolecules by optical rotatory dispersion made possible by improved instrumentation, will occupy an increasing importance in these research endeavors. There is a tremendous potential in this approach to the study of structure and function, the surface of which has only begun to be attacked. This is a major area of excitement not only in these laboratories, but around the world.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
General Metabolism

3. **AEC Budget Activity No.:**
06-04-00

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
James F. Mead

8. **Project Term:**
From: 1959 **To:** Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4	4	4
(b) Other Technical	3/4	3/4	3/4
Total	4 3/4	4 3/4	4 3/4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 39,200	$ 39,300	$ 40,400
(b) Materials & Services	6,500	6,400	6,100
(c) Indirect Expenses*	(2%) 22,400	(2%) 24,100	(2%) 24,600
Total	$68,100	$ 69,800	$71,100

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

W.G. Knipprath and J.F. Mead, the Metabolism of Trans, Trans-Octadecadienoic Acid. Incorporation of Trans, Trans-Octadecadienoic Acid into the C_{20} Polyunsaturated Acids of the Rat. J. Am. Oil Chem. Soc., 41, 437 (1964).

J.S. O'Brien, D. L. Fillerup and J.F. Mead, Quantification and Fatty Acid and Fatty Aldehyde Composition of Ethanolamine, Choline and Serine Glycerophosphatides of Human Cerebral Grey and White Matter. J. Lipid Res., 5, 329 (1964).

S. Gordon and J.F. Mead, Conversion of Linoleic-1-C^{14} Acid to Arachidonic Acid in the Gerbil. Proc. Soc. Exp. Biol. Med., 116, 730 (1964).

W.F. Libby, R. Berger, J.F. Mead, G.V. Alexander and J.F. Ross, Replacement Rates for Human Tissue from Atmospheric Radiocarbon. Science 146, 170 (1964).

J.F. Mead, Newer Aspects of Brain Lipids in Radiation Studies. Chapter 18 of "Response of the Nervous System to Ionizing Radiation", T. J. Haley and R.S. Snider, eds., Little Brown and Co., Boston, 1964.

J.F. Mead, D.R. Howton and J.C. Nevenzel, Fatty Acids, Long-Chain Alcohols and Waxes. Chapter in "Comprehensive Biochemistry", Vol. VI, M. Florkin and E.H. Stotz, eds., Elsevier Publishing Co., Amsterdam.

12. SCOPE OF THE PROJECT

Although there is no information on the substances initially affected during irradiation of tissues and living organisms, the lipids are among the prime suspects. They are readily altered by small doses of ionizing radiation and, in many cases, the products of their alteration are toxic to living organisms and may be produced by a chain mechanism which amplified the effect of the radiation. Moreover, their importance in cellular membranes and the possibility that in their ordered arrangement in such membranes, the lipids would be most readily attacked and that the results of such attack might be fatal to the cell, necessitate studies of their radiation chemistry. There is thus a need to study the primary effect of ionizing radiation on the lipids and related substances both from the point of view of the fundamental nature of the changes involved and from that of their occurrence in living organisms. The proposed studies are to investigate the alterations in unsaturated fatty acids and other lipids with irradiation both *in vitro* and *in vivo* to assess the contribution of these reactions in the overall effect of irradiation on living organisms.

Not only are the lipids suspects for a primary action of ionizing radiation on living tissues, but their importance as sources of energy and as essential metabolites makes necessary a study of alterations of their metabolism as a result of whole body irradiation. In particular, the essential fatty acids are necessary for repair of tissues following radiation or other injury and their ready susceptibility to radiation damage makes them of special interest for this type of injury. The proposed study includes a study of the function and metabolism of the essential fatty acids in their relationship to radiation injury. Also under consideration are the alterations in the brain lipids and their component fatty acids during aging and chronic low-level irradiation. The last studies are of particular importance since it is likely that

only in such relatively metabolically inert tissues as the brain will it be possible to assess the initial changes occurring with aging and low-level irradiation.

13. RELATIONSHIP TO OTHER PROJECTS

Studies in Lipid Metabolism by Means of Radioactive Tracers, H. Schlenk, U. of Minnesota. AT (11-1) 236; Studies on Lipogenesis, J.T. Van Bruggen, U. of Oregon. AT(45-1) 223; A Study of the Effect of the Diet on Lipid Metabolism Using C-14, S.B. Tove, North Carolina State College of Agriculture and Engineering. AT(40-1) 1314; The Formation and Utilization of the Saturated and "Essential" Fatty Acids in the Biosynthesis of Various Lipids in Man, S.R. Lipsky, Yale University. AT(30-1); The Effect of X-Ray Radiation on the Lipids of the Skin, H.P. Schwarz, Philadelphia General Hospital. AT(30-1) 1864; A Study of the Effect of X-Radiation of the Absorption of Glycerides Utilizing Tracer Technique, M.G. Morehouse, U. of Southern California. AT(11-1) 113; Use of Isotopic Tracers in Studies on the Nature of the Cellular Membrane and the Passage of Substances Through It, A.K. Solomon, Harvard U. AT(30-1) 609; Physiology of Serum Lipids, R.H. Turner, Tulane U. AT(40-1) 1302; Chemical Radiobiology Section; Metabolic Radiobiology Section.

14. TECHNICAL PROGRESS IN FY 1965

In a continuation of the study of the lipids of aging and irradiated brains, several young brains have been completely analyzed and differences between these and the aged brains noted in a preliminary way. Tabulation and examination of the collected data will quantitate these changes and should lead to further understanding of the aging process (see 2nd publication).

In a cooperative study of the replacement rates of human tissue constituents (see 4th publication) it was found that brain white matter (presumably myelin) protein and lipids had replacement rates not too different from those of other tissues (except collagen, which is evidently stable for at least 10 years). Since this finding does not agree with the several previous studies indicating an approximate lifetime stability for myelin, further investigation seems necessary.

In an attempt to assess the relative roles of neuronal and glial cells in production of structural lipids and energy sources, these two cell types are being separated from normal brains and from tumor material and micro-analytical methods are being developed for their study.

The mechanisms of α-oxidation of the long-chain fatty acids has been further lucidated with the finding that the unsubstituted fatty acids may be hydroxylated and decarboxylated in the presence of a brain microsomal fraction with Fe^{++} and a reduced pteridine derivative or ascorbic acid. Thus this system appears to have cofactor requirements similar to those found in several laboratories to be active in the hydroxylation of many aromatic compounds. In this connection, the metabolism of long-chain unsubstituted and α-hydroxy acids has also been studied in several strains of yeast which do not appear to possess a β-oxidation mechanism. In these cells, α-oxidation appears to be intimately connected with the chain-elongating systems leading ultimately to both even- and odd-chain unsubstituted and α-hydroxy acids with chain lengths from C_{22} to C_{27}.

In a study of the nature and function of lipids in membranes, a study has been made of the effect of environmental temperature on the structure of the fatty acids from membrane and other sources. The found increase in unsaturation accompanying decreased environmental temperature will be studied mechanistically and functionally.

15. EXPECTED RESULTS IN FY 1966

During this year, the results of the analyses of young and aging brains will be tabulated and studied and certain changes due to aging will be selected for further study. It is hoped that examples of irradiated brains will become available.

An attempt will be made to explain the seeming contradiction of a stable myelin that turns over rapidly in relationship to atmospheric CO_2. Both further human and rat experiments will be used.

The various cell types of the brain will be at least partially separated and analyzed for lipids, fatty acids, and possibly certain enzymes.

The α-oxidation system of brain microsomes will be further characterized and an attempt to clarify its relationship to the aging process will be undertaken.

The mechanism of the temperature effect on lipid structure will be studied with particular reference to the membrane lipids and the relationship between their structure and function.

A possible relationship between ionizing radiation and the aging pigment will be investigated.

16. EXPECTED RESULTS IN FY 1967

Future research will naturally depend on the results obtained from current efforts. Nevertheless, it can be anticipated that by the end of the fiscal 1967, a good understanding of the effect of aging on the human brain lipids will have been achieved. The mechanism of α-oxidation and the many influences on it, such as aging, will be better understood. The study of the function of lipids in membranes and the relationship of structure and function will have been further clarified.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
   University of California, Los Angeles

   Contract No.: AT(04-1)GEN-12

2. **Project Title:**
   Organic Chemistry

3. **AEC Budget Activity No.:**
   06-04-00

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
   Publications, UCLA Reports
   Semi-annual and Final Reports

6. **Working Location:**
   UCLA

7. **Person in Charge:**
   David R. Howton

8. **Project Term:**
   From: 1959 **To:** Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	2	2
(b) Other Technical	3/4	3/4	3/4
Total	2 3/4	2 3/4	2 3/4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 22,300	$ 27,500	$ 28,600
(b) Materials & Services	4,000	4,000	5,000
(c) Indirect Expenses*	(2%) 22,400	(2%) 24,100	(2%) 24,600
Total	$ 48,700	$ 55,600	$ 58,200

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

"Silicic Acid Chromatographic Study of the Catalytic Hydrogenation Products of 9, 10-Epoxystearates", David R. Howton and Richard W. Kaiser, Jr., Journal of Organic Chemistry, 29, 2420 (1964).

12. SCOPE OF THE PROJECT

The resources of the Organic Chemistry Section are directed toward improving understanding of changes produced in lipids by ionizing radiation. Since lipids exist in tissue in condensed phases and in intimate content with aqueous phases, both direct and indirect effects are of immediate interest. Inasmuch as the usually unbranched saturated or cis-unsaturated hydrocarbon chains they contain are largely responsible for the characteristic physical and chemical properties of the lipids (and must also be intimately involved in the physiological function of these substances), related substances containing such groups serve as models for the envisaged studies.

Insight into the course and mechanism of changes in lipids initiated by ionizing radiation (with and without the mediation of active fragments resulting from ionization of water) is expected to be provided by isolation of products and determination of their structures. Silicic acid column adsorption chromatography will be employed extensively as a key technique in the otherwise technically formidable task of isolating products from starting materials of this kind and from one another. Once isolated, the structures of these products are to be determined by study of their infrared and ultraviolet light absorption and by unequivocal degradative techniques. Fundamental studies of adsorption and light absorption phenomenon and of chemical degradation procedures will, of course, be directly pertinent.

It is anticipated that investigation of simplified model systems will serve to reveal the types of change produced by the impingement of ionizing radiation and thus make possible more facile interpretation of analogous alterations wrought in more complex systems, including particularly the phospholipid-rich membranous structures of tissue.

13. RELATIONSHIP TO OTHER PROJECTS

Los Alamos Scientific Laboratory (H.W. Langham); "Behavior of Cell Membrane Mechanisms During and Following Gamma Radiation", G.H. Bourne, Emory University, "Effect of γ-Radiation on Lipid-Protein Complexes", E.J. Hawrylewicz, Armour Res. Foundation, Ill. Tech.; "Effect of X-Ray Radiation on Lipids of Skin" H.P. Schwarz, Phila. Gen. Hospital; and studies at the Unilever Research Laboratory (England) under direction of Dr. Dennis Chapman (Head, Molecular Biophysics Unit).

14. TECHNICAL PROGRESS IN FY 1965

In connection with consideration of the mechanistic significance of the observation that the γ-radiation-induced decarboxylation (as indicated by hydrocarbon yield) of stearic acid is much more extensive (over 10-fold in the solid state) than that of oleic acid, the possibility that decarboxylation might be even more effectively suppressed when the center of unsaturation is closer to the carboxyl group has been explored by study of irradiation of trans-2-octa decenoic acid (I) under the same conditions.

Synthesis of the desired positional isomer of oleic acid by the procedure of Myers (J. Am. Chem. Soc., 73, 2100 /1951/) has been improved considerably (principally by application of silicic acid column chromatographic techniques) in order to obtain product of adquate purity. Myers' synthesis, involving Hell-Volhard-Zelinsky bromination of stearic acid (II) and successive treatment of the resulting crude α-bromostearic acid (III) with potassium iodide (to yield the α-iodo analog) and potassium hydroxide in ethanol, yields a product shown by silicic-acid-column and gas chromotography to contain 25% unchanged II, 29% α-hydroxystearic acid (IV), and 24% α-ethoxystearic acid (V), and 22% of the desired product (I) (including small amounts of the 3-isomer). Unchanged II (which would be difficult to separate from I by adsorption chromatographic and/or recrystallization techniques) is conveniently eliminated by puridication (by crystallization) of the intermediate III. After this is treated with KI and KOH in ethanol, a mixture of I (53% yield from III) and IV (16%) is easily secured (leaving the noncrystallizing V (23%) behind) by crystallization of the crude product. I is then easily and cleanly freed of IV by chromatography of the corresponding methyl esters on the silicic acid column, and saponification yields I, m.p. 58-58.5°, shown by gas chromatography to be 98.5% pure, contaminated only by less than 1% each of II and the 3-isomer of I.

Exposure of a 500-mg. sample of I to 10^8 r (6×10^{21} ev/g.) of Co^{60} γ-radiation produced a decrease in specific ultraviolet light absorption (characteristic of the α,β-unsaturated carboxylic acid grouping) corresponding to a fairly normal G_{-1} 5.95. After treatment with diazomethane (to convert all residual carboxy to carbomethoxy groups), the product was charomaographed on a column of silicic acid to yield 1.4 mg. of hydrocarbon, 386 mg. of material possessing chromatographic behavior indistinguishable from that of I methyl ester (gas chromatography of this fraction indicated that small amounts of stearic acid had been formed during the irradiation), and 68.5 mg. of more strongly adsorbed (polymeric ?) material. The yield (G 0.29) of hydrocarbon (representative of the extent of decarboxylation) did not differ significantly from that (0.27) obtained from oleic acid under the same conditions. Although the near identity of these values is probably fortuitous, it is clear that closeness of the unsaturation center to the carboxy group does not greatly alter the extent of suppression of decarboxylation. Gas-chromatographic analysis of the hydrocarbon fraction (before and after catalytic hydrogenation) showed it to be about 36% monomeric (i.e., of chain length C_{17} and shorter, the remainder being presumably dimeric), containing 58.5% 1-heptadecene (the "normal" decarboxylation product of I), 36.5% n-heptadecane (presumably formed from the other by hydrogen atom scavenging processes), and traces of lower homologs.

15. EXPECTED RESULTS IN FY 1966

Studies of the consequences of γ-irradiation of fatty acids are to be continued with the intention of establishing more definitively the chemistry of ion radicals of the sort left by ejection of an electron from substances of this kind. Investigation is expected to proceed along any or all of the following lines: 1) determination of the efficiency of intermolecular electron-deficiency transfer by investigation of yields of n-heptadecene obtained from stearic acid irradiated in presence of varied amounts of n-hexadecane; 2) attempts to elucidate the mechanism of radiation-induced decarboxylation of saturated fatty acids by study of effects of presence of free-radical scavenging agents (e.g., iodine), by establishing the structure of polymeric products formed during such decarboxylations, and by study of closely analagous

reactions of established mechanism, such as those produced by thermal decomposition of long-chain fatty acyl peroxides in the presence of homologens fatty acids; and 3) reinvestigation of studies reported several years ago on yields of radiation-induced saturated fatty acid decarboxylation as a function of chain length.

16. EXPECTED RESULTS IN FY 1967

Although it is difficult at best to attempt predicting two years in advance what progress will have been made or what new directions will have been taken by research of the sort which occupies the time and effort of This Section, it seems fairly certain that effects of ionizing radiation on lipids and related substances will still be imperfectly understood, that some intriguing mysteries will yet remain, and that the Organic Chemistry Section will therefore still be pursuing studies lying within this broadly categorized field of investigation. Providing knowledge of the radiation chemistry of free fatty acids has been advanced to a satisfactory position, it is anticipated that attention will be turned first to simple esters of fatty acids, since it is in this form that the fatty acid moieties most commonly occur in natural lipids; and nest to more complex exters, such as the phospholipids, the importance of which as membrane components makes the alteration of such substances by ionizing radiation a subject of very considerable interest.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Tracer Synthesis

3. AEC Budget Activity No.:
06-04-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Judd C. Nevenzel

8. Project Term:
From: 1962 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	2	2
(b) Other Technical	3/4	3/4	3/4
Total	2 3/4	2 3/4	2 3/4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 24,500	$ 28,000	$ 30,200
(b) Materials & Services	3,100	3,100	3,100
(c) Indirect Expenses*	(2%) 22,400	(2%) 24,100	(2%) 24,600
Total	$ 50,000	$ 55,200	$ 57,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

The Tracer Synthesis Section was established to prepare isotopically labelled molecules of interest in the biological or chemical investigations of the Biochemistry Division. To date various naturally-occurring fatty acids have been labelled with carbon-14 by techniques developed in this Division; in fact, the carbon-14 labelled unsaturated fatty acids now available commercially are prepared by modifications of our methods.

A second field of interest is the metabolism of the non-glycerol lipids (i.e., the waxes) in fish and higher plants, with particular emphasis on their biogenesis. The initial phase (still in progress) has been the accumulation of data about the occurrences and composition of the waxes; it necessarily has involved the development and testing of new methods for the extraction, analysis, and separation of wax constituents. We have used adsorption column, thin layer, and gas liquid chromatography for the separation and analysis of such constituents. Comprehensive investigations can be carried out with a few hundred milligrams of wax - an amount obtainable from a few plants or a few grams of fish tissue. The next phase will be the selection of promising species for in vivo experiments with carbon-14 or tritium labelled substrates in order to trace the pathways involved in the biosynthesis of the wax constituents, and to follow their metabolism in general. Techniques will be developed for the controlled chemical degradation of the labelled waxes.

The results of this investigation of the waxes is expected to provide basic knowledge for several types of lipids whose biochemistry is currently obscure. Incidental to our main objective, the project may answer such questions as what is the function of the wax esters in fish muscle? Is it their chemical properties (e.g., their higher ratio of carbon to oxygen in comparison to triglycerides) or their physical properties (e.g., their compressibility relative to that of water) which are more significant for this function? The study of the biochemistry of the constituents of leaf waxes may illuminate the study of brain lipids during aging now underway in the General Metabolism Section of this Division, since in both fields compounds of chain lengths than the usual C_{16} to C_{20} are involved, and one-carbon degradations seem to be important. Also we expect to clarify the role of the cuticle wax in the uptake through the leaves of inorganic elements (including those derived from fallout), in the resistance of the plants to attack by insects and micro-organisms, and in the regulation of water balance by the plant.

An example of the new techniques developed is a gas chromatographic method for the analysis of the α-alkyl glyceryl ethers (which have been proposed as radiation protective agents). Application of this analytical method can give detailed information about the individual homologues constituting the total glyceryl ethers from a given source, making possible more precise studies of the distribution and metabolism of this class of lipids. Extension of the method will allow the preparation of individual glyceryl ethers of high purity and free from homologues. It can also be readily adapted to the analysis of the vicinal diols and, probably, to the monoglycerides - two additional lipid classes of biochemical significance.

13. RELATIONSHIP TO OTHER PROJECTS

In the general areas of syntheses of labelled molecules, lipid biochemistry, and new methods in lipid analysis, separation, and degradation the Tracer Synthesis Section works closely with the General Metabolism and Organic Chemistry Sections of the Biochemistry Division.

Studies on plant cuticle waxes are in progress in the Department of Botany, University of Arizona (E.B. Kurtz, Jr.), the University of Glasgow (G. Eglinton), Laboratoire de Biologie Vetetale, Bellvue, France (P. Maxliak), and Agriculture Research Service, USDA, Beltsville, Md. (W.A. Gentner).

Investigations on the glyceryl ethers are being conducted in many laboratories, but specific interest in their effects in irradiation leucopenia is most active at ORINS (F. Snyder; AT(04-1)GEN-13).

14. TECHNICAL PROGRESS IN FY 1965

During this period the analysis of the lipids of the muscle, liver, spleen, heart, hepatic caecum, and gonads of Lepidocybium flavo-brunum and of the white muscle and liver of the "castor oil fish", Ruvettus pretiosus, both of the family Gempylidae, have been completed, and work on a fresh specimen of the coelacanth, Latimeria chalumnae (white muscle, liver, spleen, and swim bladder) was begun. In all three species the muscle contained massive amounts of lipid (15-20% wet weight basis) which was largely wax esters (88-93%) of the total lipid. The characteristic wax esters present were C_{34} with one double bond (45-60%) and C_{36} with two double bonds (15-20%). On hydrolysis wax alcohols C_{14} and C_{20} and Acids of C_{14} and C_{26} chain length were obtained. The alcohols were predominantly saturated while the acids were predominantly mono-unsaturated with only about 1% of poly-unsaturated components. In these fish we apparently find the deposition in the muscle of large amounts of wax esters which swamp the basic 1-2% (wet weight basis) of normal triglyceride-phospholipid-sterol ester lipid to be expected in this tissue. Why wax esters rather than triglycerides are deposited is not known, nor can anything definite be said about the function of this lipid.

The organ lipids were more variable, and all the data are not yet available. For example, in the livers of Ruvettus and Lepidocybium only a few percent (of the total lipid) was wax esters, but the Latimeria liver lipid is largely wax esters (based on preliminary thin layer chromatography). Other organs which contained major amounts of wax esters are Lepidocybium spleen (75%), hepatic caecum (60%) and gonads (17%) and Latimeria swim bladder (95%). The Lepidocybium heart contained no wax esters. Contrary to a previous report (Cox and Reid, J. Am. Chem. Soc., 54, 220 (1932), no hydroxy fatty acids were found in the Ruvettus pretiosus muscle lipids. The Ruvettus data have been submitted for publication.

As a consequence of concentrating on the fish samples little further progress was made in FY 1965 with plant waxes. A thin layer technique using Silica Gel G as adsorbent and 50% benzene in petroleum ether as developer which gives good resolution of aldehydes, ketones and esters, including wax esters; it was used to survey 25 plant waxes; 18 contained free alcohols, 17 aldehydes, 14 esters, and 7 ketones. The aldehydes are probably of much wider occurrence than previously thought.

15. EXPECTED RESULTS IN FY 1966

The analyses of the Latimeria chalumnae tissue lipids will be completed to increase our knowledge of this rare fish. It is hoped that through cooperation with local ichthyologists and oceanographers other fish, particularly deep-sea fish, will be available for lipid analyses. A survey of many more species is essential both for an understanding of the role of the wax esters in fish muscle, and to identify promising experimental animals for laboratory studies of the biosynthesis and metabolism of the wax esters.

Techniques must still be explored for the separation of the straight chain components of plant leaf waxes from branched (including cyclic) components; this is particularly critical for the alcohols and ketones, since sterols and terpenes are expected to be universal constituents of the leaf waxes. Chromatography on columns of a molecular sieve will be tested initially. The method developed will be applied to the analysis of the alcohol and ketone portions of the rubber plant, Ficus elastica; the free acids from this wax will be isolated and analyzed by techniques developed for the fish lipids. Labelling experiments using rooted leaves of F. elastica and acetate-C^{14} will be started, with primary interest in the incorporation into the long chain aldehydes.

16. EXPECTED RESULTS IN FY 1967

In FY 1967 we hope to be able to compare the biosynthesis of the long chain alcohols in fish and in plants. In the plant field wheat offers two advantages for this purpose; the alcohols constitute 47% of the total wax, and radiation-produced mutants with altered wax metabolism are known. Studies with some of these mutants are expected to illuminate the pathway of biosynthesis of the alcohols, and thus to strengthen our understanding of the specific genetic effects produced by ionizing radiation. It is hoped that a collaboration with a group working on the genetics of the radiation-produced wheat mutants can be arranged, since the facilities for growing the plants on the scale necessary for the isolation of the few mutants with the desired characteristics are not available at this Laboratory.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
Pathology

3. **AEC Budget Activity No.:**
06-04-00

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
T.G. Hennessy, M.D. (Acting)

8. **Project Term:**
From: 1947 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3	3	3
(b) Other Technical	½	½	½
Total	3 ½	3 ½	3 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 25,600	$ 24,200	$ 25,300
(b) Materials & Services	2,000	2,000	2,000
(c) Indirect Expenses*	(2%) 20,400	(2%) 21,900	(2%) 22,400
Total	$ 48,000	$48,100	$49,700

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

The general field of the Pathology Division research program at present is collaborative research with the other Divisions of the Laboratory, primarily in the fields of late effects of irradiation and toxicological studies. The Pathology Division furnishes the histological preparations for all research programs that may require them.

13. RELATIONSHIP TO OTHER PROJECTS

All projects of the Laboratory requiring Pathology services.

14. TECHNICAL PROGRESS IN FY 1965

Routine Pathology preparation and services have been furnished to the Laboratory during this fiscal year.

15. EXPECTED RESULTS IN FY 1966

The Pathology services will continue essentially unchanged.

16. EXPECTED RESULTS IN FY 1967

No major change in level of activity is anticipated.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
Chemical Radiobiology - I

3. **AEC Budget Activity No.:**
06-04-00

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
Lawrence S. Myers, Jr.

8. **Project Term:**
From: 1947 **To:** Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	6 ½	6 ½	7
(b) Other Technical	½	½	1
Total	7	7	8

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 59,900	$ 66,000	$ 76,500
(b) Materials & Services	9,500	9,500	11,600
(c) Indirect Expenses* (4%)	43,100	(5%) 57,000	(5%) 58,400
Total	$112,500	$132,500	$146,500

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

(1) Commentary on the Conversion of the OH· Free Radical to Cl· Atoms by Cl^- Ion. L.S. Myers, Jr. Disc. Faraday Soc. 36, 318 (1963) (Publ. 1964).
(2) Commentary on the Reactions of the OH· Free Radical (or O^- Ion Radical) in Strongly Alkaline Solutions. Disc. Faraday Soc. 36, 319 (1963) (Publ. 1964).
(3) Sites of Radiolytic Attack on Pyrimidine Compounds in Aqueous Alkaline Solution. L.S. Myers, Jr., W. T. Tsukamoto, J.F. Ward, and D.E. Holmes. Radiation Research 22, 219 (1964) (Abstract).
(4) Radiolysis of Thymine in Aqueous Solutions: Change in Site of Attack with pH. L.S. Myers, Jr., J.F. Ward, W.T. Tsukamoto, D.E. Holmes, and J.R. Julca, Science (Accepted for publication).
(5) Destruction of Thymine Dimer in Ice by γ-Radiation. L.S. Myers, Jr., W.T. Tsukamoto, and D.E. Holmes. Absts. 9th Annual Meeting of the Biophysics Society, Feb. 24-26, 1965.

12. SCOPE OF PROJECT

Information about the action of radiation on simple molecules and macromolecules is essential for an understanding of the effects of radiation on living organisms and for rational development of means of modifying these effects. Accordingly, the mechanisms by which radiation produces chemical changes are being investigated with emphasis on systems likely to give information pertinent to biology. Substances suitable for these studies include many inorganic, simple organic, and macromolecular compounds. They may be in the solid or pure liquid state, dissolved in solution, dispersed as colloids, spread as monolayers, or organized into membranes, depending on the type of mechanism or reaction under investigation. Attempts to apply information gained from these and related studies to biological systems are continually under way. This section is also responsible for the Laboratory Radiation facility.

13. RELATIONSHIP TO OTHER PROJECTS

This work is generally related to investigations in this Laboratory under J.F. Ward, J.F. Mead, D.R. Howton, and G.V. Taplin.
Many other laboratories throughout the world are conducting related studies in radiation chemistry and biochemistry.
Those most closely related are:

B. Ekert, Institut du Radium, Fondation Curie, Paris, France; Joseph Weiss, University of Newcastle-upon-Tyne, England, Bert Tolbert, University of Colorado, Boulder, Colorado; William H. Prusoff and Paul Howard-Flanders, Yale University, New Haven, Connecticut; and E. Pollard, S.E. Person, and W. Ginoza, Pennsylvania State University.
Others include: National Institutes of Health; University of California, Davis, Berkeley, and Los Angeles; University of Notre Dame; Oak Ridge National Laboratory; Argonne National Laboratory; Brookhaven National Laboratory, and several laboratories in England, France, Australia, Sweden, and Russia.

14. TECHNICAL PROGRESS IN FY 1965

During this year studies of radiation effects on some of the constituents of nucleoproteins and on related model compounds have been continued. Substantial progress was made as follows:

Effects of Ionizing Radiation on Thymine in Dilute Aqueous Solutions.
Previous work has shown that the site of radiolytic attack on thymine in aqueous solutions shifts from the 5,6 double bond to the 5-methyl group as the pH is increased through and beyond the physiological region. It was also shown that attack on the methyl group leads to formation of an oxidation product tentatively indentified as 5-hydroxymethyl uracil (HMU). However the possibilities that the product might be 5-formyluracil or uracil-5-carboxylic acid were not excluded. Further investigations, largely by means of paper chromatography and radioautography of carbon-14 labeled materials, have definitely excluded these latter compounds and have confirmed that the product is indeed HMU. In addition considerable progress has been made on determining the yields of products resulting both from attack on the double bond and on the methyl group as a function of pH, with the aim of developing a mechanism for the reaction and an explanation for the pH dependence.

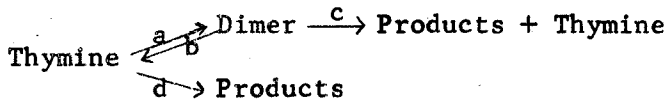
Effects of Ionizing Radiation on a Model Compound (Toluene) in Aqueous Solutions
Under pH conditions such that radiolysis of aqueous solutions of thymine leads to formation of 5-hydroxymethyl uracil, the attacking species is the $O^{\cdot -}$ radical ion, and the pyrimidine ring of thymine is aromatic. To determine whether $O^{\cdot -}$ reacts in general with methyl substituted aromatic compounds to form hydroxymethyl derivatives, toluene was irradiated in dilute alkaline (attacking species $O^{\cdot -}$) and neutral (attacking species OH·) aqueous solutions, and the products were determined by gas chromatography. The product analogous with HMU would be benzyl alcohol. Higher oxidation products would be benzaldehyde and benzoic acid, and reaction with the aromatic ring would give cresols. Products formed by $O^{\cdot -}$ were benzaldehyde and benzyl alcohol in the ratio 6 to 1. No cresols were found. Products formed by OH· were benzaldehyde, cresol, and benzyl alcohol in the ratio 1 to 1 to 1. Thus reaction of $O^{\cdot -}$ with the methyl group of a methyl substituted aromatic compound appears to be a general reaction; the product however is not necessarily the lowest oxidation state (the hydroxy compound). The OH· radical, on the other hand shows an appreciable tendency to react with the aromatic ring.

Destruction of Thymine Dimer in Ice by γ-Radiation

Under conditions favorable for formation of the thymine dimer in ice by ultra-violet radiation, the dimer appears to be destroyed by ionizing radiation. Dimer was prepared by freezing a 2×10^{-4} M solution of thymine and exposing it to ultra-violet light from an H23KX lamp. While still in the original ice it was irradiated at -79° by γ-rays from a 10,000 curie cobalt-60 source. After irradiation the solutions were melted and ultra-violet absorption spectra were obtained. For successively larger irradiation doses the optical density of the 263 mμ peak increased slightly, then decreased. γ-irradiation of frozen thymine solutions resulted only in a decrease. These results suggest that on γ-irradiation of the dimer two reactions are occurring: a) release of a molecule containing an intact thymine-like chromophoric group, and b) destruction of the chromophoric group. Yields for the two reactions, based on energy absorbed in the entire system, are small ($G < 0.02$) and approximately equal.

The lability of the dimer in the presence of γ-radiation does not necessarily prove that the dimer is not formed by γ -irradiation. Analysis of the products in γ-irradiated frozen thymine solutions indicates to the contrary that the major initial product is the dimer. A lesser product, which is also formed in liquid aqueous solutions, appears to be the result of saturating the 5,6 double bond by free radical attack, and may be a dihydroxy derivative of thymine.

Combining these two sets of observations suggests the following overall scheme for the action of γ-radiation on thymine in frozen aqueous solutions



Reactions a and b appear to be the result of excitation processes, and c and d of free radical reactions, largely the OH· free radical. The relative importance of b vs c is not yet known.

Preliminary Investigations of Radiation Effects on Nucleic Acid Constituents

Because of the possibility that the direct action of radiation is important biologically, and also to aid in interpreting experiments such as those described above with ice, dry films of thymine, thymidine, and uridine have been irradiated. The samples were then dissolved and analyzed by ultra-violet spectrophotometry and paper chromatography. In all three compounds the chromophoric group was attacked, and several products were formed. The extent of the reaction was much greater when the films were exposed to oxygen than to nitrogen.

An investigation of the reactions of hydrogen and deuterium atoms with nucleic acid constituents has been initiated. The atoms are generated in a vacuum system and allowed to react with highly dispersed material in the cavity of an electron paramagnetic resonance spectrometer. Results indicate that reaction of H· or D· with adenine, adenosine, adenylic acid, and polyadenlic acid results in the same organic free radical, i.e. the reaction is with the adenine part of the compound, and appears to involve abstraction rather than addition of an atom. This work was carried out in cooperation with Dr. Ronald B. Ingalls at Atomics International Division of North American Aviation, Co.

Initial steps have been taken towards investigating the initial radiation induced reactions on nucleic acids and nucleoproteins in vitro and in vivo. Labeled nucleic acids have been grown in E. Coli, and techniques are being developed for isolating and identifying the radiation products.

Development of the Radiation Facility

Continued progress has been made in developing the 10,000 curie cobalt-60 source as a versatile tool for use in the Radiobiological Sciences. In addition, operation of 250 kvp and 50 kvp x-ray units and a 150 curie cobalt source for specialized experiments has been continued.

15. EXPECTED RESULTS IN FY 1966

Investigations of the mechanisms of the radiolysis of biologically important compounds will continue with emphasis on the nucleoproteins and their constituents. Further investigations of radiation effects on thymine will be continued in an attempt to develop a reaction mechanism which takes into account both pH and oxygen effects, and to gain a greater understanding of the relative importance of direct and indirect action of radiation and of energy transfer processes. Considerable work is also planned on other pyrimidines and purines and their nucleosides and nucleotides to determine the effects of various side chains. The greatest emphasis will probably be on polynucleotides and nucleic acids with the aim of determining the initial products of the action of ionizing radiation, and the importance of these reactions in biology. The availability of an EPR spectrometer will greatly

facilitate studies of free radical reactions on all of these compounds. Investigations of the action of radiation on appropriate model compounds will also be carried out if it appears that such studies will be helpful in interpreting results of any of the preceding experiments. Efforts to develop further the 10,000 curie cobalt-60 source as a versatile tool for radiobiological research and operation of the Laboratory's other radiation sources will be continued.

16. EXPECTED RESULTS IN FY 1967

A continuing effort will be made to obtain information about radiation effects at the molecular level which will provide a basis for understanding the biological effects of radiation. Studies of nucleoproteins, their constituents, and related compounds will undoubtedly continue to be of primary importance. The particular problems to be investigated and possible consideration of other types of biologically important compounds will depend on the results of the preceding year's work.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No,: AT(04-1)GEN-12

2. Project Title:
Chemical Radiobiology - II

3. AEC Budget Activity No.:
06-04-00

4. Date Prepared:
April , 196

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
John F. Ward

8. Project Term:
From: 1964 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	1 ½	2 ½	3
(b) Other Tech.	½	½	1
Total	2	3	4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 18,300	$ 23,600	$ 27,500
(b) Materials & Services	4,000	4,100	5,600
(c) Indirect Expenses* (1%)	12,200 (1%)	13,300 (2%)	24,600
Total	$ 34,500	$ 41,000	$ 57,700

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

(1) Effect of Bromide Ions on the Destruction by γ-rays of Nucleic Acids and Their Constituents in Aqueous Solution. J.F. Ward and S.J. Hadsall. Radiation Research 22, 248 (1964).

(2) Sites of Radiolytic Attack on Pyrimidine Compounds in Aqueous Solution. L.S. Myers, Jr., W.T. Tsukamoto, J.F. Ward, and D.E. Holmes. Radiation Research 22, 219 (1964)

(3) γ-Irradiation of Oxygenated Aqueous Cysteine Solutions. J.F. Ward. Absts. 9th Ann. Meeting of the Biophysical Society, Feb. 24-26, 1965, p. W.F. 3.

12. SCOPE OF THE PROJECT

γ-irradiation damage to a living cell obviously involves many possible mechanisms. This project is concerned with the measurement of damage produced in important biological molecules "in vivo" and "in vitro". The mechanisms of damage to nucleoproteins, transforming principle DNA and their constituent molecules is being investigated. It is necessary to try to relate the chemical changes produced in simple systems to the biological effects observed in order to know how these changes can be modified.

13. RELATIONSHIP TO OTHER PROJECTS

This work is related to the investigations carried out under the direction of L.S. Myers, Jr., J.F. Mead, D.R. Howton and R.D. Lehman in this Laboratory. Studies in a similar vein are being carried out throughout the world. Most closely related:

J.J. Weiss, University of Newcastle-upon-Tyne, England; P. Howard-Flanders, Department of Radiology, Yale University, New Haven, Connecticut; A. Pihl, Norsk Hydro Institute for Cancer Research, Montebello, Oslo, Norway; K.G. Zimmer, Institüt für Strahlenbiologie, Kernforschungrzentrum, Karlaruhe, Germany; F. Hutchinson, Department of Molecular Biology and Biophysics, Yale University, New Haven, Connecticut.

Others include: National Institutes of Health, University of California, Berkeley and Los Angeles, University of Notre Dame, Brookhaven National Laboratory; and several laboratories in England, Russia, France, New Zealand, Australia, Sweden and Japan.

14. TECHNICAL PROGRESS IN FY 1965

Effect of Chloride Ion on some Radiation Chemical Reactions

Thymine and ethanol compete for OH free radicals produced by irradiation of water. In the presence of sodium chloride in acid solution a different reacting species is produced which reacts preferentially with thymine. By varying the pH and chloride ion concentration independently it is shown that the production of this species depends on both the hydrogen ion and the chloride ion concentration.

Further investigation involving more accurate determinations of the variable parameters and yields, and a careful calculation of the data gives a rate equation which can be satisfied by a novel mechanism. This mechanism involves the reaction of H_2O^+ with Cl^-, the life time of H_2O^+ being extended by its production in the reaction of OH with H_3O^+. Heretofore H_2O^+ was supposed to have no definite significance in radiation chemical mechanisms, but the extension of its life time by the reaction of OH with H_3O^+ shows that its reactions can not be neglected.

γ-Irradiation of Aqueous Cysteine Solutions

Further studies of this system has enabled a complete stoichiometric analysis of the reactions taking part to be obtained. Sensitive analysis of radiation products by chromatography of S^{35} cysteine (after blocking the -SH group with N ethyl maleimide) was carried out. This confirmed previous suspicions that cysteine was not the major primary radiation product. The main primary radiation product is cysteine sulfinic acid which is then reduced by excess cysteine to cysteine. The complete radiation chemical scheme then is

$$\xrightarrow{\gamma \text{ rays}} \quad H_2O \rightsquigarrow H,\ e_{aq},\ OH,\ H_2,\ H_2O_2$$

$$RSH + H \longrightarrow RS^{\cdot} + H_2 \qquad H_2S$$

$$RSH + e_{aq} \longrightarrow RSH^{-} \rightarrow SH^{-} + R^{\cdot}$$

$$RSH + OH \longrightarrow RS^{\cdot} + H_2O$$

$$RS^{\cdot} + O_2 \longrightarrow RSO_2^{\cdot}$$

$$RSO_2^{\cdot} + RSH \longrightarrow RSO_2H + RS^{\cdot}$$

$$RS + RS \longrightarrow RSSR$$

$$RSO_2H + 3RSH \longrightarrow 2RSSR + 2H_2O$$

It has also been shown that secondary free radicals will attack cysteine, i.e. the reaction of a thymine molecule with an OH free radical produces a hydroxy thymine radical which can thereafter react with cysteine.

Hence the importance of cysteine in radiation biochemistry is shown in two ways: (a) it can react readily with other free radicals produced in the system, forming the sulfinic acid in the presence of oxygen and (b) this sulfinic acid can further react with other sulfhydryl groups in the system.

15. EXPECTED RESULTS IN FY 1966

Preliminary work with DNA and bacteria in which the mechanism of various protectants was examined will be pursued in 1965. It is hoped that the electron paramagnetic resonance spectrometer can be used to study the free radicals produced in cells irradiated at -170°C, the effect of the above mentioned protectors on this system will be investigated. It may be possible to study the mechanisms of protection by warming the system and allowing the original free radicals to move around.

Further work on purine systems will also be carried out with a view to obtaining evidence as to their relative importance in determing the radiosensitivity of DNA.

16. EXPECTED RESULTS IN FY 1967

The study of free radical effects in cellular systems will be further investigated in the manner described above. It may be necessary to use other sources of free radicals such as a hydrogen atom generator or OH free radicals produced by the photolysis of hydrogen peroxide. Use will be made of methods of modifying the radiosensitivity of systems. Concomitant experiments on simple molecular systems will be necessary in all cases.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Metabolic Radiobiology

3. AEC Budget Activity No.:
06-04-00

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Ole Arne Schjeide

8. Project Term:
From: 1952 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	5	6	6
(b) Other Tech.	1	1	1
Total	6	7	7

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 48,200	$ 61,800	$ 63,100
(b) Materials & Services	14,000	14,600	14,600
(c) Indirect Expenses* (4%)	42,900	(4%) 46,000	(4%) 46,900
Total	$105,100	$122,400	$124,600

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

(1) Phospholipid Synthesis in the Nucleus of the Erythrocyte. O.A. Schjeide, R. Marshall, M. Wilkens, R.C. McCandless and R.J. Munn. Nature 202, 33-36 (1964)

(2) Transfer Ribonucleic Acids. E.N. Carlsen, G.J. Trelle and O.A. Schjeide. Nature 202, 984-986 (1964)

(3) Mitochondrial Morphogenesis. O.A. Schjeide, R.G. McCandless and R.J. Munn. Nature 203, 158-160 (1964)

(4) An Ultrastructure Study of Pityrosporum Orbiculare. M. Barfatani, R.J. Munn and O.A. Schjeide. J. Invest. Dermatol. 43, 231-236 (1964).

(5) Nuclear-Cytoplasmic Interactions. O.A. Schjeide, Ruth G. McCandless and R.J. Munn. Nature 205, 156-158 (1965)

(6) Biochemical and Morphological Aspects of Radiation Inhibition of Myelin Formation. O.A. Schjeide, J. Yamazaki, Karen Haack, Elizabeth Ciminelli and C.D. Clemente. Acta Radiologica (1965). Appearing in Special Supplement.

(7) Ultracentrifugal Assay of Zinc Binding in the Proteins of Chicken Eggs and Serum. J.P. OKunewick, O A. Schjeide, and G. Glancy. Biophysical Journal 5, 35 (1965) (Abstracts).

(8) Macromolecular Parameters of Estrogen Induced Protein Synthesis. Gary Lai and O A. Schjeide. Biophysical Journal 5, 157 (1965) (Abstracts).

(9) Composition of Myelin in Irradiated Rats. O.A. Schjeide, K. Haack, J. de Vellis, J. Yamazaki, C.D. Clemente. Radiation Research 24, (1965) (Abstracts).

12. SCOPE OF THE PROJECT

The purpose of the Metabolic Radiobiology Section is to study the modifications of intracellular metabolic mechanisms by ionizing radiations, to determine how these changes are brought about and to relate the effects to the total organism. The Developmental Physiology Unit is primarily concerned with radiation effects on metabolic systems at different levels of differentiation.

Aside from studying problems that deal strictly with applications of radiation this section has been, and will be concerned with phenomena that are basic to the knowledge of biological systems in general, since it is reasoned that such information may ultimately prove to be of considerable assistance in the elucidation of radiation-induced effects and to the amelioration of such disturbances.

Specific Areas of Investigation:

(1) Chemical and enzymatic analysis of developing myelin in brain stems of irradiated neo-natal rats. Special attention is being given to the lipid and protein composition of the myelin, and to possible alterations in concentrations of selected enzymes following irradiation.

(2) Studies of regulation of synthesis of cell organelles and materials by genetic mechanisms. This includes isolation and chemical and enzymatic analysis of materials observed to pass from nucleus to cytoplasm.

(3) Studies of regulation of synthesis of proteins by genetic mechanisms. This includes: isolation and characterization of messenger and carrier RNAs during induction of phosphoprotein synthesis by estrogen treatment.

(4) Studies on synthetic mechanisms of the nuclei of circulating non-dividing red cells in chicken embryos are being continued including synthesis of hemoglobin, lipids, soluble lipoproteins, messenger and carrier RNA, DNA, and enzymes associated with oxidative phosphorylation.

(5) Chemical and enzymatic analysis of organelles from cells of embroys and oocytes following their isolation by density gradient centrifugation. Whenever possible the effects of radiations on the various metabolic processes listed above are assessed and in most cases, the studies are carried out on tissues at different levels of differentiation.

As these problems are being attacked, occasions arise that call for the development or elucidation of technical aspects of the research. Some deviations in these directions have been made and will continue to be made.

13. RELATIONSHIP TO OTHER PROJECTS

This work is related to studies of radiation induced derangements of intracellular components, serum proteins, lipids, and lipoproteins which are being carried out on adult organisms in the Biochemistry Division of this Laboratory, and similar investigations being carried out at Rochester, Argonne and Oak Ridge.

The most unique feature of these investigations is that they are being performed on developing cell systems.

14. TECHNICAL PROGRESS IN FY 1965

During 1965 the Section has accomplished the following:

Passage Across the Nuclear Membrane

Electron micrographs of rapidly differentiating embryo cells indicate that the so-called "pores" of the nuclear membrane are usually not open holes through which materials from nucleus and cytoplasm may exchange willy-nilly. Tangential cuts across the surface of the nucleus reveal that a dense granule is consistently fixed in the centers of each of these. (The roles of the granules are not known.)

How then, do materials pass from nucleus to cytoplasm and vice versa? A common mode of transfer of nuclear materials (these may include information for cytoplasmic syntheses) to the cytoplasm is by disassociation of vesicles arriving at the "pore" site on the nuclear side into very much smaller vesicles and the re-emergence of the smaller vesicles on the cytoplasmic side which re-assemble into multi-vesicular bodies of characteristic organization. The periphery of the "pore" site appears to be involved in passage of the micro-vesicles. The multi-vesicular bodies have been observed to be associated with protein and organelle syntheses in the cytoplasm. They appear to be able to pass from cell to cell, traversing the two limiting membranes by a dissassembly-reassembly process similar to that described for passage across the nuclear membrane.

At least some vesicles approaching the nuclear membrane are observed to be present as such in the nucleolus. Here they are sometimes arranged in double helical coils that can be seen to be unraveling into two strands of vesicles. Some of the vesicles appear to arrive at the nuclear membrane still arranged in strands.

Radiation-Inhibition of Myelin Formation

Non-lethal doses of X-irradiation (750 roentgens) administered to the heads of new born rats (2 days of age) result in failure of the brains to attain normal sizes. The departure from normal in the brain stem (the most primitive part of the brain and the first part to attain maturity) takes place at approximately two weeks of age. This is the point in development when certain highly specialized cells, the neuroglial cells, begin to lay down

layers of insulating material around the actual nerve fibers in the form of fatty sheaths (myelin). Pictures taken with the electron microscope reveal that the myelin is indeed thinner or absent from the nerve tracts of the irradiated animals but that the myelinating cells are not destroyed, only slowed in their growth and development. In attempting to determine the biochemical reasons for this retardation, it has been found that animals irradiated as late as 6 days of age are not inhibited in their deposition of myelin. Certain enzyme deficiencies which have been found in animals irradiated at 2 days of age, such as those which convert the fat stearic acid to oleic acid (oleic acid is prominent in myelin), are also not found in animals irradiated at a later age.

These findings lead us to suspect that some of the enzyme systems which are responsible for making myelin are laid down in the cytoplasm of the neuroglial cells at about 2-4 days of age (even though formation of myelin does not take place at a rapid rate until two weeks of age). The process of establishing such systems appears to be radio sensitive but once the enzymes are made, as at 6 days of age, irradiation seems to have little effect. Support for this thesis comes from findings showing that enzymes which appear prominently in the brain stem before 2 days of age including those involved in glycolysis, are actually increased proportionately in the cytoplasms of neuroglial cells as a consequence of irradiation.

Animals receiving irradiation to the head show losses in body weight, muscle spasms and incoordinations which cannot be ameliorated by growth hormone or thyroxin. Irradiations of the body (500r) but with the head shielded results in a small rat with a relatively large head and no retardation of myelination or nervous symptoms. Although differences between irradiated and control rats are not as great in rats allowed to live for longer periods, complete recovery is never achieved by the head-irradiated animal.

In Vitro Synthesis of Hemoglobin by Nucleated Red Cells

It has been demonstrated that circulating avian red cells from both embryos and adults incubated with C^{14} lysine will incorporate this isotope not only into hematin but into the globin fraction as well. The uptake into globin in the embryo cells (13 days of incubation) is approximately 10 times faster than in the cells from chicks indicating a much more rapid synthesis of globin in cells from embryos. Experiments are being carried out at the present time to determine if any of the globin is synthesized in the nuclei from which it may be subsequently passed to the cytoplasm. It has already been demonstrated that much hematin is indeed synthesized in the nucleus and is passed to the cytoplasm.

Ribonuclease Digestion of Transfer RNA from Livers of Control and Estrogenized Roosters

As much as one third of the total transfer RNA from livers of estrogenized roosters may consist of carriers that are specific for serine and phosphoserine. RNAase digests of transfer RNAs from control and estrogenized livers have been compared. Predominant nucleotides in the control transfer RNA appear to Be C, GC, and U with lesser amounts of AC, AU, GU, AGU and/ or GAU and uracil-5 ribosyl phosphate. In sRNA from livers of heavily estrogenized roosters larger proportions of AC, AU, GU, AGU and/or GAU and methyl guanidine are present. Quantitation of these results, by neutron activation of the "fingerprints" of these fractions, is being carried out.

Morphology of the Developing Avian Red Cell

Developing red cells in the liver of the 6-day-old chicken embryo have been studied. Features of paramount interest include: 1. Nucleo-cytoplasmic pores that are not open "holes" as in circulating red cells. 2. The presence of strings of vesicles within the chromatin bodies of the nuclei. 3. Prominent ribosome clusters within the cytoplasm. These are far less frequent in circulating cells. 4. Well developed cristae in the mitochondria as compared with the degenerate-appearing cristae in the mitochondria of circulating cells. 5. Pinocytosis in free floating cells. 6. Exchange connections between developing red cells and liver cells. 7. Prominent rows of vesicles or tubules arrayed at cytoplasmic tips.

Distribution of Firmly Bound Zinc in the Serum and Egg of the Laying Hen

Although it has previously been shown that zinc is bound equally (on a weight basis) among all of the serum proteins of the rat, in the serum of the laying hen Zn^{65} has been shown by studies involving differential ultracentrifugation to associate predominantly with serum phosvitin, a protein that has been postulated to be a precursor for egg yolk phosvitin. Serum phosvitin is never present in the serums of rats or normal roosters. It is induced by estrogens in egg laying species and contains in high proportion of phosphorus as does egg yolk phosvitin. The lipid-phosphorus containing lipoproteins bind less zinc than do non-lipoproteins.

The Zn^{65} present in serum phosvitin is not lost during the incorporation of this protein into the egg yolk. Egg yolk phosvitin binds more than 65 times more Zn than do the proteins which constitute the "livetin" fraction of egg yolk.

Occurrence, Synthesis and Transport of "Mitochondria-like" Organelles of Avian Oocytes

Organelles of approximately the same size as the smaller mitochondria but distinquishable from these chiefly in that they do not display typical cristae, are formed in considerable numbers just inside the surfaces of follicular cells surrounding the developing egg. They possess a double external envelope (exclusive of the plasma membrane that is closely applied about the organelle in its final form) with membranes of about 40-50 angstroms each in thickness. In addiation, there are small regulary spaced projections from the internal membrane into the interior of the organelle, the stalks of which terminate in an electron dense knob of about 100 angstroms in diameter. It is not known if any of the enzymes associated with respiration and oxidative phosphorylation are present in these organelles.

Their formation at the surface of the follicular cell and their ultimate locations suggest that they are formed in response to come stimulus from the oocyte that has diffused to the site of synthesis. Their synthesis is always associated with the conversion of that portion of the limiting membrane that will bound the developing organelle, into microvesicles. These microvesicles begin at one point and spread laterally. At the same time micro- and larger-vesicles elaborate inward to produce the double membrane of the organelle and the stalks and knobs of the regular projections from the internal membrane into the relatively fluid interior of the organelle.

As the length of double membrane being synthesized increases the process begins to bud into the intercellular space. Finally this bud pinches off and the completed organelles are found in groups in the intercellular spaces.

Subsequently they pass to the oocyte where they enter vesicles which become - or join - yolk granules. The role of the organelles therein is not known but it is recalled that in frog and turtle oocytes an association between conventional appearing mitochondria and the developing yolk also exists.

Incorporation of Substrate into Nucleated Red Cells

Rates of uptake of C^{14} glycine, C^{14} lysine, C^{14} cysteine, C^{14} cystine, C^{14} tryptophane, C^{14} glucose and C^{14} acetate have been compared in red cells from embryos and from chicks. Generally speaking, uptake of all of the above substrates, with the exception of glucose, is several times faster in cells from embryos than in those from chicks. Glucose is taken up at a rate that is almost equal in the two types of cells. Substrates are incorporated at rates that are correlated with their molecular weights. Most of the incorporation is due to active - rather than passive - transport. Studies are being carried out to determine if rates of transport through the membranes are limiting steps in the rates of incorporation of substrates into cell materials. Activity of certain enzymes is also being ascertained.

15. EXPECTED RESULTS IN FY 1966

During 1966 studies will be carried out on the following:
(1) Nucleocytoplasmic exchanges in various cells following various treatments; isolation and characterization of nucleus derived vesicles; (2) Further characterization of nucleic acids in estrogen treated animals; (3) Isolation and characterization of mitochondria-like organelles; isolation and characterization of intra-mitochondrial inclusions; (4) Nucleic acid, enzyme, and lipid changes in isolated myelin from irradiated brain stems of neo-natal rats.

16. EXPECTED RESULTS IN FY 1967

Continuation of 1966 studies with emphasis on (1) biochemical aspects of nuclear syntheses in normal and irradiated cells (2) isolation and characterization of subcellular organelles (3) enzyme and nucleic acid changes in irradiated brain stems of neo-natal rats.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Cellular Radiobiology

3. AEC Budget Activity No.:
06-04-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Noel de Terra Whittaker

8. Project Term:
From: 1961 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3	3 ½	5
(b) Other Technical	1	1	1
Total	4	4 ½	6

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 24,400	$ 33,000	$ 48,200
(b) Materials & Services	3,500	3,800	5,600
(c) Indirect Expenses*	(1%) 12,200	(2%) 24,100	(3%) 35,800
Total	$ 40,100	$ 60,900	$ 89,600

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Nucleocytoplasmic Interactions during the Differentiation of Oral Structures in Stentor coeruleus. Noël de Terra. Developmental Biology 10. 269-288, (1964).

12. SCOPE OF THE PROJECT

A study of the physiological and biochemical mechanisms by which specific cell activities are regulated in unicellular organisms, with special emphasis on the interactions of nucleus and cytoplasm. Although a great deal has been learned in recent years about the biochemical regulatory mechanisms of bacteria, relatively little is known about the regulatory mechanisms of protozoa or of metazoan cells. It is felt that studies of this kind will lead to a more complete understanding of important cell activities such as cyto-differentiation and will also provide valuable background information for studies of abnormal cell growth and function.

The regulatory mechanisms of unicellular organisms are currently being investigated by experimental studies on cytoplasmic control of nuclear morphology and function in the ciliate Stentor, regulatory mechanisms governing regeneration or oral structures in Stentor and synthesis and transport of digestive enzymes in Amoeba proteus.

Techniques in current use include cell micrurgy (enucleation of cells, transplantation of nuclei), radioautography and histochemistry.

13. RELATIONSHIP TO OTHER PROJECTS

Cell regulatory mechanisms are now the subject of intensive study in many laboratories around the world. The following workers are currently investigating the regulatory mechanisms of unicellular organisms: T.M. Sonneborn, University of Indiana, Bloomington; Jean Brachet, Universite Libre de Bruxelles, Belgium; O.H. Scherbaum, University of California, Los Angeles; Lester Goldstein, University of Pennsylvania, Philadelphia; Vance Tartar, University of Washington, Seattle; Konrad Keck, Johns Hopkins University, Baltimore; George Brawerman, College of Physicians and Surgeons, New York.

14. TECHNICAL PROGRESS IN 1965

Synthesis of Ciliary Protein in Stentor coeruleus

Regeneration of oral structures in Stentor involves the formation of the many thousand cilia comprising the oral membranelles. One would like to know whether the formation of these cilia involves the de novo synthesis of ciliary protein or the assembly of pre-formed protein molecules. An extremely sensitive method for assessing incorporation of labelled precursors into the oral membranelles has been developed during the past year and successfully used to demonstrate synthesis of ciliary protein during regeneration. In this method, the oral apparatus is isolated from the cell before radioautography by treatment with 2 percent urea, which induces shedding of the membranellar band without apparent harm to the cells. The membranelles can thus be viewed against the negligibly labelled photographic emulsion rather than against the heavily labelled ground cytoplasm characteristic of sections or whole cell squashes.

Using this technique, appreciable label has been found over the cilia of membranelles isolated from cells which regenerated their oral structures immediately after a brief exposure to tritiated leucine. It is therefore

concluded that at least some of the protein used for construction of the oral membranelles during regeneration is synthesized during the process of regeneration itself. This work provides an explanation for the well-known dependence of regeneration in Stentor on the presence of the cell nucleus.

Further experiments have shown that tritiated leucine is also incorporated into the oral membranelles of stentors which are not regenerating. This incorporation presumably reflects the synthesis of new cilia as the oral apparatus increases in size during the cell growth cycle. This result strongly suggests that the induction of regeneration does not depend on activation of previously quiescent genes concerned with the synthesis of ciliary protein.

Preliminary Studies on the Role of the Cell Nucleus in Intracellular Digestion

In protozoa, intracellular digestion is known to be immediately dependent on the presence of the cell nucleus. Recent work suggests that intracellular digestion is accomplished through transfer of digestive enzymes from cytoplasmic organelles called lysosomes into food vacuoles. It would be of great interest to know whether the presence of the cell nucleus is required for transfer of lysosomal enzymes into food vacuoles. Since enucleate cells will not ingest food organisms, it is difficult to approach this problem experimentally. However, it has been reported that enucleate amoeba will ingest protein solutions by the process of pinocytosis or cell-drinking. If it could be proved that pinocytosis vacuoles are a type of food vacuole, it would become possible to study the role of the cell nucleus in digestion by comparing the migration of a lysosomal enzyme into pinocytosis vacuoles of nucleate and enucleate amoebae.

In order to determine whether or not digestion occurs within pinocytosis vacuoles, amoebae (A. proteus) were starved to free them of food vacuoles and immersed in a ferritin solution to induce pinocytosis. Groups of cells were fixed at various time intervals thereafter and stained both for ferritin and for acid phosphatase, a lysosomal enzyme. Immediately after removal of the amoebae from ferritin, acid phosphatase was found to be localized in the lysosomes, while the ferritin was present in large vacuoles. Six hours later, the ferritin-containing vacuoles were also found to contain acid phosphatase. It therefore appears that pinocytosis vacuoles fill with lysosomal enzymes and are, in fact, a type of food vacuole. Future experiments will deal with the migration of acid phosphatase into pinocytosis vacuoles of nucleate and enucleate amoebae.

15. EXPECTED RESULTS IN FY 1966

Investigation of regulatory mechanisms in unicellular organisms will continue, with emphasis on the study of mechanisms regulating DNA synthesis in Stentor.

Since it is possible to transfer nuclei from cell to cell in Stentor, this organism provides an exceptionally favorable opportunity for experimental analysis of factors controlling nuclear DNA synthesis during the cell cycle. Nuclear transfer and other micrurgical techniques will be used in combination with autoradiographic studies of tritiated thymidine incorporation into nuclear DNA to investigate questions such as the following: Is nuclear DNA synthesis turned on and off during the cell cycle by cytoplasmic events? Does the occurrence of DNA synthesis depend on removal of an inhibitory influence or on the appearance of a previously missing factor? Are the mechanisms regulating DNA synthesis in Stentor at all similar to the mechanisms which regulate DNA synthesis in bacteria?

16. EXPECTED RESULTS IN FY 1967

In 1967, more emphasis will be placed on investigating the relative roles of structural rearrangement and macromolecular synthesis in the regulatory mechanisms of protozoa. When a cell activity such as nuclear DNA synthesis in Stentor or intracellular digestion in Amoeba is initiated, does this occur because components which are already present have been rearranged or structurally altered in some way or because a previously missing element has been synthesized? This problem will be approached by using the techniques of cell micrurgy, radioautography, histochemistry and enzymology.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Physical Radiobiology

3. AEC Budget Activity No.:
06-04-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
E. Hardin Strickland

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	3	4
(b) Other Technical	½	½	½
Total	2 ½	3 ½	4 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 16,600	$ 31,200	$ 37,700
(b) Materials & Services	4,400	4,500	5,900
(c) Indirect Expenses*	(1%) 12,200	(2%) 24,100	(2%) 24,600
Total	$ 33,200	$ 59,800	$ 68,200

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. E.H. Strickland and C.R. Goucher. Effects of Ferric Nucleotides on Mitochondrial Respiration. Arch. Biochem. Biophysics 108, 72-84 (1964).

12. SCOPE OF THE PROJECT

This project is concerned with cellular metabolic pathways and their regulation. Special emphasis is placed on aerobic metabolism and the energetics of adenosine triphosphate (ATP) reactions. ATP plays a primary role in many metabolic reactions that must be driven by energy-yielding reactions. In most cells the mitochondria are the principal site of ATP formation. The factors which influence the turnover of ATP are being investigated in order to gain a fuller understanding of the mechanisms of the reactions and eventually to measure the rates of these reactions within intact cells. These studies on the regulation of cellular metabolism will provide basic information which will facilitate understanding the damaging effects of ionizing radiation upon tissue.

13. RELATIONSHIP TO OTHER PROJECTS

Since studies on the control of phosphorylative reactions are one of the leading lines of current biochemical and biophysical endeavor, related work is being pursued in numerous laboratories, e.g., under Professor Britton Chance at the University of Pennsylvania and under Professor Lester Packer at the University of California. Furthermore, the current project will include an investigation of respiratory and phosphorylative activities of cultured heart cells, done in collaboration with Dr. Issac Harary (Biochemistry Division, Laboratory of Nuclear Medicine and Radiation Biology).

14. TECHNICAL PROGRESS IN FY 1965

Mitochondria contain significant quantities of non-heme iron. Green and co-workers have presented evidence that non-heme iron functions in mitochondrial electron transport. Whether or not non-heme iron functions in the phosphorylative reactions remains uncertain.

Investigations of the interaction between mitochondria and exogenous iron have been hindered by the low solubility of ferric hydroxide, about 10^{-15} M at pH 7, and by the rapid oxidation of ferrous iron in the presence of oxygen. The existence of a variety of ferric nucleotides, which are soluble at physiological hydrogen ion concentrations, suggested a new approach for the study of non-heme iron in oxidative phosphorylation. Investigations reported last year showed that ferric nucleotides are able to stimulate succinate oxidation to a higher level than magnesium plus nucleotides. Evidence was obtained suggesting that this stimulatory effect might be due to mitochondrial iron binding.

Iron Binding by Mitochondria

Experiments in this laboratory have shown that mitochondria accumulate iron when incubated in a medium containing a ferric nucleotide. The mitochondrial iron binding, however, differs from the accumulation of divalent cations (Ca^{++}, Mg^{++}, Ba^{++} and Mn^{++}) reported by other investigators, because iron uptake appears to occur by an energy-independent mechanism. Iron uptake probably reflects binding to a mitochondrial component such as a protein or nucleic acid. The ability of mitochondria to accumulate large quantities of iron suggests that iron temporarily stored on mitochondrial binding sites may be utilized for the synthesis of iron-containing proteins, e.g., respiratory chain enzymes.

Oscillatory Enzymatic Reactions

Recently Chance and co-workers described damped oscillations of glycolytic intermediates in anaerobic yeast. These investigators suggested that oscillatory enzymatic reactions were a reflection of a product activating a prior enzymatic step in the glycolytic pathway. As a consequence of this conclusion, enzymatic oscillations can be used to identify sites of metabolic control.

In view of this, it is of fundamental importance to determine if oscillations can occur only in enzyme systems having inhibition or activation of enzymes by other reactants or whether, on the contrary, most enzymatic reactions can oscillate. A theoretical investigation carried out in this laboratory showed that any single enzymatic reaction involving two or more enzyme-substrate complexes may have kinetic equations characteristic of damped oscillations, if the kinetic constants satisfy certain conditions. However, these oscillations are so highly damped that they probably cannot be observed experimentally. Thus it appears that oscillating enzymatic reactions do reflect enzyme activation and inhibition in a metabolic pathway.

15. EXPECTED RESULTS IN FY 1966

The regulation of phosphorylative reactions in heart cells cultured in vitro will be investigated. The effects of ion transport, protein biosynthesis, contraction and other energy requiring processes will be investigated by measuring the response of pyridine nucleotide fluorescence.

Related investigations will involve the mechanism of action of certain ATP-dependent enzymes. Optical rotatory dispersion will be used to investigate possible conformation changes in enzymes during the catalytic process.

16. EXPECTED RESULTS IN FY 1967

Fluorescence studies will be extended to include an investigation of phosphorylative reactions in other cell types. More emphasis will be given to studies of enzyme conformation and its relation to function.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
Soil Factors

3. **AEC Budget Activity No.:**
06-05-01

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
H. Nishita

8. **Project Term:**
From: 1959 **To:** Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4	4	4
(b) Other Technical	1/4	1/2	1/2
Total	4 1/4	4 1/2	4 1/2

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 39,900	$ 38,500	$ 39,700
(b) Materials & Services	3,600	3,700	3,800
(c) Indirect Expenses* (3%)	33,200 (2%)	21,900 (2%)	22,400
Total	$ 76,700	$ 64,100	$ 65,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Influence of stable Sr and Ca on Sr90 and Ca45 in soils and clay minerals. Soil Sci. 98: 181-186. September 1964.

2. Release and movement of radionuclides in soils contaminated with fallout material from an underground thermonuclear detonation. Health Physics: Accepted for publication, 1964.

12. SCOPE OF THE PROJECT

The objectives of the Soil Factors Section are geared to one of the objectives of Environmental Radiation Division to study the biological cycling of radionuclides released into the environment as a result of utilization of nuclear energy. Accordingly, this Section is concerned with soil-plant interrelationship problems with particular emphasis on the chemistry of fission products and neutron induced radionuclides in soils. Presently, this Section is involved in three projects:

(1) Influence of chelating agents on the reactions of fission products in plants and soils.
(2) Effect of Neutron irradiation of soils.
(3) Influence of certain clay minerals on the uptake of Sr90 and Cs137 by plants.

The scope and the objectives of these projects are discussed below in the "Expected Results" section.

13. RELATIONSHIP TO OTHER PROJECTS

Plant Factors, Nuclear Medicine and Radiation Biology, E. M. Romney.

Environmental Decay, Nuclear Medicine and Radiation Biology, H.A. Hawthorne.

University of California, Berkeley, California AT(11-1)-34 #23.

U. S. Department of Agriculture, Beltsville, Maryland AT(49-7)1.

University of Tennessee, Knoxville, Tennessee AT(40-1)2077.

Ecology Section, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

14. TECHNICAL PROGRESS IN FY 1965

Further investigations into the effects of chelating agents (EDTA, DTPA, and EDDHA) on the movement of the radionuclides Sr90, Y91, Ru106, Cs137, and Ce144 in soil columns are in progress. Previous work was concerned only with a contaminated calcareous soil. The work in progress is a study of the influence of chelating agents on different kinds of soils (acidic, neutral, calcareous, and organic). Available information indicated that EDTA and DTPA increased the movement of Y91 in all the soils studied. The greatest movement of Y91 occurred in the neutral Vina loam and the least in the acid soil. The movement of Ru106 occurred in all soils, but its movement appeared to be due only to the movement of water. The chelating agents did not have any appreciable influence. Cesium137 did not move under the influence of these chelating agents.

Radioactive decay characteristics of several different soils activated by neutron irradiation in the UCLA Engineering Nuclear Reactor were studied. Theoretical decay characteristics of soils of assumed elemental composition also were studied by calculation on a computer. Although the six reactor activated soils differed widely in chemical properties from an agronomic point

of view, the general trends of their decay curves were quite similar up to 10,000 hours. The result implied that the elemental composition of these soils was similar. The differences in the absolute amounts of each element in the soils were manifested by the differences in the activity levels per unit weight of the soils and the slopes of the decay curves at a specified time. The radioactive decay rates were relatively rapid to about 200 hours after irradiation and then changed to noticeably slower rates. Depending on the time after irradiation, the decay constants of the experimental soils varied between $T^{-0.2}$ and $T^{-5.0}$. Although a number of radionuclides may be formed, the decay curves were dominated by relatively few.

15. EXPECTED RESULTS IN FY 1966

(1) Influence of chelating agents on the reactions of fission products in plants and soils.

The objectives of this project are to study the influence of chelating agents (DTPA, CDTA, EDDHA, and EDTA) on the reactions and movements of Y91, Sr90, Zn65, Cs137, Ru106, Ce144, and Pm147 in soil columns. As stated above, previous work has been done only with a calcareous soil. The present work is intended to compare the effectiveness of different chelating agents in different soil types --acid, neutral, alkaline-calcareous, and organic soils.

(2) Effect of neutron irradiation of soils.

The objectives of this project are to study the effect of neutron irradiation of several clay minerals and soils of different physical and chemical properties. These materials are irradiated in the UCLA Engineering Reactor and examined for the following: (1) the gross beta and gamma decay rates of the neutron induced activity, (2) the water soluble, exchangeable, and acid soluble fractions of induced activity in the soils, (3) availability of induced activity to plants, and (4) change in physical and chemical characteristics as a result of irradiation. Work on Item 1 has been completed and a report is under preparation. Presently, the major part of our effort is concerned with Items 2 and 3.

(3) Influence of certain clay minerals on the uptake of Sr90 and Cs137 by plants.

One of the means of reducing the biological cycling of radionuclides that might be dispersed in the biosphere is to fix these nuclides in soils and reduce their availability to plants and to animals that ingest certain amounts of soil in foraging. To accomplish this, it is necessary to find means to selectively fix hazardous radionuclides in soils. With this purpose in mind, this project proposes to study the influence of certain clay minerals on the plant uptake of Sr90 and Cs137 from contaminated soils. Presently, experiments are in progress to study the influence of the use of clinoptilite as soil amendment on the plant uptake of Sr90 and Cs137.

16. EXPECTED RESULTS IN FY 1967

Certain phases of the projects in progress during FY 1966 will be continued. It is anticipated that the studies on the influence of chelating agents on the movement of certain radionuclides in soil columns will be completed and a project to study the influence of certain chelating agents on the translocation of fission products within the plants will be initiated, as well as a study of the change in physical and chemical characteristics of soils as a result of gamma and neutron irradiation.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Plant Factors

3. AEC Budget Activity No.:
06-05-01

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
E. M. Romney

8. Project Term:
From: 1953 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4	4	4
(b) Other Tech.	1/4	1/2	1/2
Total	4 1/4	4 1/2	4 1/2

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 40,300	$ 40,700	$ 43,900
(b) Materials & Services	3,400	4,700	5,400
(c) Indirect Expenses*	33,200 (3%)	21,900 (2%)	33,600 (3%)
Total	$ 76,900	$ 67,300	$ 82,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Potassium and magnesium relations in trifoliate orange seedlings grown in sand culture. Amer. Soc. Hort. Sci. Proc. (in press)(with A.H. Khadr et. al.) 1965 .

2. Effects of beryllium in plants and soils. Soil Science (accepted for publication) 1965.

3. Effects of chelated iron and zinc on rough lemon and trifoliate orange seedlings grown in calcareous soil. (with A. Wallace, et. al.) Submitted to Amer. Soc. Hort. Sci. Proc. 1965.

4. Mineral nutritional peculiarities of trifoliate orange (with A. Wallace, et. al.) Submitted to Calif. Agric. 1965.

12. SCOPE OF THE PROJECT

The research program of the Plant Factors Section is twofold in scope: (1) to improve understanding of the functions of plants in transferring radioactive contaminants and toxic mineral elements through the soil to plant link of food chains leading to animals and man, and (2) to investigate the mineral nutritional and the physiological characteristics and pecularities of native species of desert plants in collaboration with ecological studies being conducted by other investigators of the Environmental Radiation Division.

The objectives aim to a better understanding of fundamental mechanisms involved in the uptake and distribution of fission products, neutron-induced radionuclides, and toxic mineral elements; and their subsequent influence on physiological characteristics of plants. Included are studies on (1) the reactions of these materials in soil, (2) the environmental and physiological factors influencing their absorption and distribution by plants, and (3) the deleterious effects to plants resulting from the absorbed radionuclides and toxic mineral elements.

Investigations are conducted in controlled laboratory environments and in the field in order to help bridge the gap between practical and theoretical problems associated with environmental contamination. Results from this research program should assist in establishing parameters for assessing the consequences to man of radioactive contaminants and toxic mineral elements in the environment, and increase knowledge of the nutritional and physiological characteristics and peculiarities of desert plants.

13. RELATIONSHIP TO OTHER PROJECTS

Research is correlated with the Soil Factors, Plant Physiology, Environmental Radiation and Ecology Sections of the Environmental Radiation Division.

Related studies are conducted at the following laboratories:

Biology Department, Battelle-Northwest, Richland, Washington
Ecology Section, ORNL, Oak Ridge, Tennessee
Univ. of California, Berkeley, California
U. S. Dept. of Agric., Beltsville, Maryland
University of California, Riverside, California

14. TECHNICAL PROGRESS IN FY 1965

Continued studies on the influence of O_2 supply to roots on plant uptake of fission products and essential mineral nutrients disclosed that Sr90 uptake was reduced, but Cs137 uptake was increased, by decreasing the O_2 supply to roots of sudan grass and soybeans grown in soil and nutrient solution. Decreasing the O_2 supply to roots reduced plant uptake of calcium, potassium and phosphorus, but did not alter sodium uptake. Applications of NH_4 in place of NO_3 as the only source of nitrogen supply to plant roots significantly reduced the uptake of Ca45, Sr85 and 90, Cs137, and Ce144 by soybean plants. Biochemical analyses are in progress to investigate changes in plant metabolism which might be caused by altering the O_2 and CO_2 content of the root environment. Current emphasis is being placed on the organic and amino acid constituents as indices of these effects.

Findings from continued studies of Be-toxicity in plants showed that Be must be soluble in order to inhibit growth, and that direct contact of plant roots with insoluble Be-salts is not a significant mechanism involved in inhibiting plant growth. In reactions with soil, Be showed a strong chemical resemblance to aluminum which enabled it to undergo isomorphic substitution in the secondary clay minerals in addition to cation exchange reactions. Be was strongly fixed in soil and it displaced other divalent cations, including Ca, Mg and Sr, which shared common sorption sites. Be-treatments of 4-8 ppm in nutrient solution reduced $C^{14}O_2$ assimilation by bush bean plants. The assimilated C14 was distributed among the neutral, anion and cation constituent fractions in the ratio of 3:3:1, irrespective of the Be-treatment levels, after exposure of Be-treated beans to $C^{14}O_2$ for 1 hour. The relative distribution of C14 among the major amino acid constituents of the cation fraction was similar for both Be-treated and control plants. Amino acids quantitatively identified by paper chromatography were asparagine, asparatic acid, glutamic acid, alanine and threonine. Only qualitative identification could be made for lysine, and phenylalanine. Analyses to determine C14 distribution among the organic acids and neutral organic constituents of Be-treated plants are in progress.

Investigations of the reactions in soil and the availability to plants of neutron-induced Sc46, Sb124, Ta182 and W185 showed that Sc46 and Ta182 were strongly fixed in soil in forms that were relatively unavailable to plants. Very little movement of Sc46 and Ta182 occurred in soil subjected to heavy leaching. With the exception of acidic types of soil in which high fixation occurred, Sb124 moved freely in soil and was readily available to plants. Reactions of W185 were similar to Sb124, but less movement occurred in soil subjected to heavy leaching. Fixation and exchange reactions of Sb124, Ta182 and W185 were influenced by type of clay mineral, soil pH, and the concentration of alkali metal cations present in the soil.

15. EXPECTED RESULTS IN FY 1966

(1) Beryllium effects on plants

Studies of deleterious effects of Be on plant metabolism will be continued with emphasis being placed on the organic and amino acid constituents as indicators of inhibiting effects. Interactions between Be and the essential mineral nutrients, Ca, Mg and P, will continue to be investigated.

(2) Influence of root environment on radionuclide uptake by plants

Studies will continue to investigate the effects of altering the O_2 and CO_2 concentration in the root environment of higher plants on the absorption and distribution of radionuclides, and to investigate any concomitant changes in plant metabolism. This project will involve collaborative work with the Plant Physiology Section.

(3) Reactions in soil and availability to plants of radionuclides

It is anticipated that laboratory investigations of reactions of Sc46, Sb124, Ta182 and W185 in soil and their uptake by plants will be completed by FY 1966 unless additional activities occur in the area of peaceful applications of nuclear energy which would necessitate extension of this project. Uptake of neutron-induced radionuclides and fission products by native desert plants will continue to be studied in conjunction with investigations of the mineral nutritional and physiological characteristics and peculiarities of desert plants which will begin during FY 1966.

16. EXPECTED RESULTS IN FY 1967

Certain phases of research projects in progress during FY 1967 will be continued. We anticipate that histological studies to investigate the inhibiting effects of Be on root development may be initiated during FY 1967 since this project had to be deferred until the technical capabilities could be developed for this kind of work. Investigations of the mineral, nutritional and physiological characteristics and peculiarities of desert plants at the NTS Ecology study areas will be emphasized during FY 1967.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles

   Contract No.: AT(04-1)GEN-12

2. **Project Title:**
   Environmental Decay

3. **AEC Budget Activity No.:**
   06-05-01

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
   Publications, UCLA Reports
   Semi-annual and Final Reports

6. **Working Location:**
   UCLA, Southern Utah, Nevada Test Site

7. **Person in Charge:**
   H. A. Hawthorne

8. **Project Term:**
   From: 1957 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	5	5	5
(b) Other Technical	1	½	½
Total	6	5 ½	5 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 49,700	$48,400	$ 49,500
(b) Materials & Services	12,900	13,500	5,400
(c) Indirect Expenses* (4%)	43,500 (3%)	32,900 (3%)	33,600
Total	$106,100	$94,800	$ 88,500

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Fission product cycles in an agricultural system. I. Sample heterogeneity. 2nd AEC Conference on Radioactive Fallout from Nuclear Weapons Tests. TID-7701 (1965). H. A. Hawthorne.

2. An inexpensive resistance welder for thermocouple fabrication. TID-19268 (1964). R. A. McDonough and H.A. Hawthorne.

3. A visual null-point indicator for a soil moisture bridge. Agron. J. 56: 244 (1964). H.A. Hawthorne and R.A. McDonough.

4. Thermal fusion of thermocouples by internal heating. H.A. Hawthorne and R.A. McDonough. (Submitted for publication).

5. Contamination of plant foliage with radioactive fallout. E.M. Romney, R.G. Lindberg, H.A. Hawthorne, F.G. Bystrom and K.H. Larson. (Abstract). Biol. Abstr. 45: 9494 (1964).

12. SCOPE OF THE PROJECT

The purpose of this project is to investigate the cycling of the fission products Sr-90, Cs-137 and Ce-144 in a dairy farm where the feeds required in the production of milk are grown on that farm. The objective of these studies is to construct mathematical models of the movements of the fission products as they occur under the restrictions imposed by the particular edaphic, climatic and management practices present at the farm.

Two kinds of mathematical models are involved. In the first, a deterministic model, the average or mean rates of transfer of the fission products between the soil, plant and animal components of the farm are determined. In the second model, a stochastic model, the movements of the fission products are treated as random occurrences subject to probabilistic laws. The stochastic model uses the variations in fission product distributions among the farm components as input from which the probability of very large or very small concentrations of fission products can be specified. The conversion of deterministic models to the stochastic mode requires that large numbers of radiochemical determinations be made so that the variation in fission product distributions can be established for each of the components of the farm or system. Consequently, deterministic models for each of the fission products are made as a preliminary to development of stochastic models, since mean values can be obtained from small samples without fear that the means will change appreciably when more analytical determinations are made. Small samples, six to eight specimens, are analyzed for their fission product concentrations and the data is used with a computer program to estimate the numbers of additional fission product determinations required to provide the precision and confidence intervals needed in the stochastic models.

The technical approach can be simply stated: All additions, losses, rates of fission product transfers, the biologically active pools, and the quantities of fission products residing in the system are accounted for. Execution of the program is most complex.

Fallout deposition is measured, the proportions retained by crops is ascertained, and the biological availability of the fission products with plants is determined from the percentage of dietary Sr-90, Cs-137 and Ce-144 synthesized into milk. The proportions of fission products taken from the soil in root uptake by plants is determined by isotopic ratios in combination with

laboratory studies. Plants are taken from the farm with soil around their root systems and propagated in a growth chamber where external contamination is prevented. The size of the biologically active pool of fission products in the soil can be derived from the rates and quantity of plant uptake. The availability of fission products retained by plants used for dairy feeds is elicited by feeding trials. All the materials fed to livestock are weighed for a 10 day period, the milk produced is weighed, samples of drinking water, feeds, milk and feces are collected and their fission product concentrations are measured. A feeding trial is run each time there is a significant change in the diet of the cattle, for example, the feeding of a different cutting of alfalfa or the addition of silage to the diet.

Farm management practices are studied for their effects upon rates of cycling of fission products and the changes they cause in the size of the biologically active pool of isotopes. The characteristics of the soil and plants at the farm are carefully defined so that their influence upon transfer coefficients is learned. Irrigation water and runoff are sampled to determine the amounts of radionuclides left with the soil. Fluctuations in climatic variables are recorded so the effects of their changes upon plant growth and root uptake can be interpreted.

The results of the field studies are extended in their application by using the soils and plants in laboratory experiments with other soils and plants. Comparisons of the response of materials from the farm to materials with different characteristics enables predictions to be made about the movements of fission products in different environments. Information about how fission products respond to a known set of parameters through an entire food chain from the atmosphere to human foods is needed in the prediction of fallout hazards. During periods of low stratospheric fallout deposition, information can be derived about root uptake from fallout created by weapons tests made at the Nevada Test Site since 1951, and later combined with that from stratospheric fallout from the large weapons when this is the main source of plant contamination.

13. RELATIONSHIP TO OTHER PROJECTS

Field studies of food chain relationships in dairy operations are in progress at Colorado State University, Fort Collins, Colorado, under the supervision of Dr. Gerald Ward and at the University of Michigan, Ann Arbor, Michigan, by Dr. G.W. Whipple. Studies are conducted at the Laboratory of Nuclear Medicine and Radiation Biology in collaboration with the Soil Factors Section and the Desert Ecology Section.

14. TECHNICAL PROGRESS IN FY 1965

Several months' use of Cobal computer programs showed that they were too inflexible to handle both data reduction and statistical analysis. The Cobal programs are being replaced by Fortran IV data reduction programs with optional subroutines for statistical evaluation of data. Eight hours of IBM 7094 computer time were used in program "debugging" and production runs. A programmer-data analyst worked eight months with the computer programs. Dr. K. H. Lu, biostatistical consultant from the University of Oregon, worked 10 days with problems of the farm studies. Methods of assessing variability of fission product concentrations in materials, field collection methods and laboratory techniques were reviewed, and procedures for determining sample size were checked. He initiated Markov chain analysis of the farm system and

worked with path analysis of the transfers of fission products in the food chain. An Analysis of Variance scheme was programmed for computer use.

Radiochemical work was restricted to processing "estimator samples", six to eight specimens per sample, to ascertain the variations in fission product concentrations in different materials from the farm. From these data the numbers of radiochemical determinations needed to give predetermined precision within selected confidence intervals were estimated for defining the amounts of fission products associated with soil, plants and dairy products at the farm. With the estimates of radiochemical analyses required, the objectives of the study can be limited to those where statistical precision is possible. This selection process has relied upon computer programs. Results to date were presented at the Second Fallout Conference, Germantown, Maryland, on November 6, 1964.

The statistical interpretation of data by an Analysis of Variance presumes that the variances for the factors involved are of equal size. This assumption was thought questionable with respect to yields of alfalfa crops produced at the farm. Variances among the weights of alfalfa per unit area of field were tested for homogeneity by a modified Bartlett's test using yields from each crop harvest in the past two years. The variances between fields were equal for each month; but the variances in production within individual fields were unequal, both with respect to years and between harvests within years. Standard deviations in yield varied with the month of the year, and between years when harvests for the same month were compared. Variations in yields were not predictable from month to month although the dates of economic maturity were predictable. The results indicated that each crop must be sampled throughout the study to obtain reliable data on yields and their variability. Limited effort was expended in determining frequency distributions for yields by individual fields but the results indicated that they can be defined. Samples from past collections, from all the fields collectively, did describe yield frequency distributions for the farm satisfactorily.

Field operations required 193 man-days for sample collection of crop production and feeding trials. Nine feeding trials, each of 10 days' duration, were required for measurement of the biological availability of the fission products associated with different cuttings and kinds of plants fed to dairy cattle. Before harvest operations, representative samples of each crop produced on the farm were collected and again after the crops were harvested. Fallout retention will be determined from the pre-harvest samples, and the additions of fission products by the harvesting operations will be measured by the differences between the sets of samples. Fallout deposition was measured by collectors maintained during the growth of each crop. Irrigation water, precipitation, drinking water, and waste water leaving the farm were sampled. An inventory of all the materials on the farm was taken in May to obtain an isotopic material balance upon which annual additions and losses of fission products could be based.

Contamination of alfalfa by fallout continued to be highest at the May cutting with lower amounts with each of the four succeeding cuttings. In May of 1963 and 1964 there were 2,200 pc Sr-90/kilogram. In May of 1964 there were 3,300 pc Cs-137/kg. compared to 1,500 in May of 1963. The retention of fallout by alfalfa plants decreased 10-fold between May and October of 1963, relative to fallout deposition. In the dairy, pronounced variations in the percentage of dietary Sr-90 and of Cs-137 metabolized into milk were observed. The cause of the differences is under study.

15. EXPECTED RESULTS IN FY 1966

This will be the last year of sample collection at the dairy farm near St. George, Utah but sample collection will represent the major effort for the year. Past experience has shown that collection, preparation of samples for storage and for radiochemical analyses will occupy the technical manpower the entire year. Processing of estimator samples to establish the number of specimens needed to define variations in fission product concentrations should be completed during this year. There will continue to be a strong emphasis upon computer usage for data reduction and sample size estimation, with need for a programmer and close consultation with a statistician.

The first complete deterministic model will be developed as the analytical results from the first year of sample collection are acquired. This model will be tested with data from the second year of sampling to check its prediction capabilities.

An emphasis upon collection of the large numbers of specimens required for determination of more sophisticated frequency distributions will be made in the crop production phases. Studies elsewhere with similar materials indicated that they probably will best be described by gamma functions, and these will be used with crop yield data. If they are found appropriate they will be extended to isotopic data in subsequent years.

A large amount of data is accumulating from the Nevada Test Site in a collaborative project with Dr. Beatley. Soil temperatures and soil moisture records are taken every week for correlation with phenological observations on desert annuals. A computer program will be completed for data reduction and statistical correlations. Transfer of the collected data to punch cards is anticipated during this year.

Preliminary work on the biochemical effects that periods of high soil moisture stress have upon plant composition will be undertaken. Testing of procedures should be completed before the close of the year.

16. EXPECTED RESULTS IN FY 1967

Specimens from the farm study will be selected to accomplish only those objectives which can be attained with statistical precision. Quite large numbers of specimens will be submitted for analyses to the Radiochemical Problems Section, and the first firm isotopic variation data on the distribution of fission products should become available in this year. As work on the farm study decreases there will be an increase in effort to define the mechanisms by which perennial shrubs at the Nevada Test Site escape desiccation during drought, as a cooperative project with the Desert Ecology Section.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Plant Physiology

3. AEC Budget Activity No.:
06-05-01

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
W. A. Rhoads

8. Project Term:
From: 1960 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3	3	3
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$
Total	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{1}{2}$

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 33,200	$ 33,000	$ 33,000
(b) Materials & Services	2,700	3,500	3,500
(c) Indirect Expenses* (2%)	22,900 (2%)	21,900 (2%)	22,400
Total	$ 58,800	$ 58,400	$ 58,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

This project proposes to investigate the fundamental physiology of roots of plants under conditions more nearly like those found in the environment of plant roots in nature, that is where CO_2 concentrations are vastly increased from that of the atmosphere and where oxygen concentrations may be either that of the atmosphere or greatly reduced. Much of the knowledge of root metabolism is known by analogy to other plant parts, or to other metabolic systems: in both cases the information comes largely from cyto-plasmic-particle research. The first objective of this project is to investigate the respiratory rates and metabolic processes of intact roots under conditions of varied environments. The first step is to determine the effects of increased CO_2 concentrations about the roots, to find whether the increased concentrations encountered may not change the paths of carbon utilization and synthesis from that found in plant tissues in "normal" atmospheric concentrations of either or both CO_2 and O_2.

The respiratory rates of roots are being studied under conditions of increased CO_2 by both volumetric analysis and by infrared spectrometry. This permits investigation of a wide range of conditions which correspond much more closely to conditions in nature than to conditions under which plants are ordinarily studied in the laboratory.

An investigation of the metabolic processes is being followed by the use of chromatography with paper and silica-columns and by the use of carbon-14 for tagging metabolites to aid in identification and analysis.

This project has as a third objective a contribution to understanding of processes in plants which affect or influence mineral utilization by plants, particularly cesium and strontium which have roles in plants analogous to the roles of potassium and calcium respectively. These studies should also contribute to understanding the mal-utilization of iron which often occurs in calcareous regions in species with such susceptabilities.

13. RELATIONSHIP TO OTHER PROJECTS

Similar projects, or projects which are working on related problems are in progress at the following institutions:

Soil-Plant Factors, Nuclear Medicine & Radiation Biology, H. Nishita
Plant Factors, Nuclear Medicine & Radiation Biology, E. M. Romney
Plant Physiology Section, Hanford AEC Operations, Richland, Washington
Utah State University, Logan, Utah
U. S. Dept. of Agriculture, Beltsville, Md.
Department of Plant Biochemistry, UCLA
Department of Hort. Sci. U. of California, Davis, California

14. TECHNICAL PROGRESS IN FY 1965

Bush bean plants were grown in constant temperature growth chambers with the temperature held at a constant 75 degrees F. Under this condition the respiratory rate of bean roots, intact, in vivo was about the same regardless of whether the plants were aerated with air with 5.40% or 3.15% carbon dioxide in air both plus or minus 0.05%. The respiratory rate with 5.40% carbon dioxide was 0.51 plus or minus 0.15 and 0.47 plus or minus 0.10 micro-liters carbon dioxide respired/minute/gram (dry weight) of roots respectively

in two experiments. In a third experiment with 3.15% carbon dioxide the rate was 0.49 plus or minus 0.07 microliters respired/minute/gram. The rates were the same in both cases in the light and in the dark.

Modification of the cell length to accommodate concentrations of carbon dioxide the root media up to 10% carbon dioxide were achieved and the system of gathering exhaust gases from the growing solution media were improved so that possible differences due to aeration rates could be detected between the rates of 15 ml aerating gas/minute and 150 ml aerating gas/minute being put into one gallon nutrient solution containing three mature bean plants. Further studies were begun on the effects of light and dark on the observable respiratory rates of plant roots.

15. EXPECTED RESULTS IN FY 1966

During FY 1966 we anticipate discovering considerable information on the respiratory rates of bean plants aerated with concentrations of carbon dioxide in air from 2.0% to 20.0%. In addition there should be some data forthcoming on the concentrations of metabolites, particularly the concentrations of the organic acids of the Kreb's cycle, found in roots under these conditions. This information should allow interpretation of the pathways of carbon utilization by beans as the results of the changes of carbon dioxide concentrations in the roots' environment. This data should provide information as to the relative importance of the known capability of plants to fix carbon by dark-fixation (non-photosynthetic) processes.

16. EXPECTED RESULTS IN FY 1967

An investigation of other plant species among those known to be tolerant of poorly-aerated soils, and those tolerant of calcareous soils will be undertaken in an attempt to better understand the adaptive mechanisms of plants and in an attempt to understand something of the different metabolic processes presumed to exist due to differences in differently-adapted species.

It is anticipated that knowledge gained in the laboratory from the preceding two years will be utilized to work with plants in the field, in order to contribute to the understanding of those species under study in other Sections of this Division, particularly those species encountered at the Nevada Test Site by the Ecologists working that region.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Radiation Ecology - Mammalian Irradiation

3. AEC Budget Activity No.: 06-05-01

4. Date Prepared: April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Norman R. French

8. Project Term:
From: 1959 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	6	6	6
(b) Other Tech.	1/4	1/2	1/2
Total	6 1/4	6 1/2	6 1/2

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 60,300	$ 60,500	$ 61,600
(b) Materials & Services	7,100	7,800	8,000
(c) Indirect Expenses* (4%)	43,500	(4%) 43,800	(4%) 44,700
Total	$ 110,900	$ 112,100	$ 114,300

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Fertility and population density of the black-tailed jack rabbit. N.R. French, R. McBride, J. Detmer. J.Wildlife Mgmt. 29: 14-26. 1965.

2. Description of a study of ecological effects on a desert area from chronic exposure to low level ionizing radiation. N.R. French. U. S. Atomic Energy Comm. Report. UCLA 12-532. 27 pp. Oct. 1964.

3. The source of ingested radioactivity in desert rodents. N. R. French, P. Hayden, T. Tagami. Health Physics (in press) 1965.

4. Analysis of dispersal in desert rodents. N.R. French. Bull. Ecological Soc. Amer. 45 (3):115. 1964 (abstract).

5. A radiation facility for ecological studies at the AEC Nevada Test Site. N. R. French. Bull. Ecological Soc. Amer. 45 (3):79-80. 1964 (abstract).

12. SCOPE OF THE PROJECT

Effects of chronic exposure to low level gamma radiation on natural populations of desert rodents are being investigated. This approach treats the population as the functional unit, and examines those attributes which are important to maintenance of the population. Such attributes are: mortality, fertility, and age structure of the population.

A field experiment is in progress at the Nevada Test Site. A portion of the natural population is enclosed in a 20 acre area, and is exposed to gamma radiation for approximately 26 days and nights each month. Rodents, marked for individual identification, are captured monthly. Records are maintained on the location and time of capture for each animal. From such records, mortality rates for different aged animals and production of young by the population can be evaluated. A control enclosure of the same size but without irradiation provides comparative data. A sample of approximately 100 rodents is taken quarterly from the free-living population of nearby regions. Animals from these samples are examined in the laboratory for age and reproductive condition. These data permit evaluation of mortality, fertility and age structure of the free-living population for comparison with the enclosed irradiated and control populations.

Laboratory studies conducted in support of the field studies include evaluation of the effect of chronic low level gamma exposure on fertility of the wild mouse Peromyscus maniculatus. Records of litter size are maintained on irradiated and control pairs, derived from the same wild breeding stock. Production of young will be evaluated by covariance analysis. Blood of control mice and mice exposed to one r/day for several months to a year is being examined. Total red cell, total white cell, and differential white cell counts will be examined by regression analysis or covariance analysis.

A laboratory study of the growth and wear of teeth of wild rodents is being conducted in an effort to improve our estimates of age. Methods of estimating metabolic rates of rodents are being examined in laboratory studies, and will later be used in the field to determine energy requirements of the rodent population.

The results will indicate whether this level of exposure can be tolerated by a population of desert rodents. Due to the known sensitivity to radiation of the rodent reproductive system, it is anticipated that the first impairment of the population will manifest itself in the production of young. The

importance of this impairment to population size and energy utilization should be indicated by the results. In cooperation with the studies being conducted by Dr. Turner on reptiles, and by Dr. Martin on plants, these results will indicate the importance to a desert ecosystem of chronic exposure to low level ionizing radiation.

13. RELATIONSHIP TO OTHER PROJECTS

Investigations of the reptiles, invertebrates and plants in the field study area (Rock Valley) are being conducted by other investigators of this Laboratory. Population studies of wild mammals are being conducted at Oak Ridge National Laboratory and Savannah River Operations. Effects of radiation on wild mice in the laboratory are being investigated at Savannah River Operations, the University of Georgia, and Northrup Space Laboratories.

14. TECHNICAL PROGRESS IN FY 1965

1. The study plot in Rock Valley at the Nevada Test Site has been irradiated for one year. Dosimetry at ground level has been conducted with E.G. & G. micro-thermoluminescent dosimeters and with shielded and unshielded radio-luminescent glass rods. The dose rate varies between 435 mr/hr at 50 feet from the center and 64 mr/hr at 550 feet, with an average dose rate of about 5 r/day. Low energy scattered radiation is detectable out to 150 feet from the center, but probably does not increase biological dose.

Micro-thermoluminescent dosimeters have been attached to rodents. Exposure of these animals depends upon their location in the radiation field and their activity time on the surface of the ground. The latter is considerably reduced during the winter months, and measured exposures indicate dose rates at this season to be less than one r/day. Census of the population at monthly intervals continues. Numbers of rodents in the enclosures and throughout the Rock Valley area remain low as a result of the poor breeding season during 1963.

2. A method that can be utilized in the field for estimating total CO_2 production by an animal over a period of days is being tested in the Laboratory. It is based upon the fact that the oxygen of body water is lost both as respiratory CO_2 and as water, while hydrogen is lost only as water. The rates of loss can be measured by isotopic enrichment of the body water with O^{18} and H^2, and quantitative determination of the degree of enrichment in blood samples taken at different times. The difference between rates of loss of these isotopes permits calculation of the expired CO_2 and evaluation of energy utilized by the animal.

The method is being tested in the Laboratory on heteromyid rodents. These conditions permit measurement of oxygen consumption and collection of the CO_2 produced by the animal, as a check on the procedure. If we are successful, the method will be used on animals in the field.

3. A laboratory population of deer mice, Peromyscus maniculatus, is receiving gamma radiation at a rate of approximately one r/day. Fertility of this colony is to be compared with fertility of the control colony. Both irradiated and control animals are descendants of the same wild stock bred in the Laboratory. Animals were paired at the time of weaning. Inbreeding was avoided. One group of experimental animals has been irradiated since weaning age (approximately 21 days), and another group since conception.

Blood tests on all irradiated and control animals are in progress. Counts are made of the total red and white blood cells, and a differential count will be made of the white blood cells.

4. A method for estimation of the age of wild rodents is under investigation. It will permit evaluation of age structure and mortality in a free-living population. Ca^{45} is utilized to indicate the degree of calcification of secondary dentin of the teeth. A quantitative measure of calcification should be correlated with age.

Certain factors influencing Ca^{45} uptake by sections of teeth have been examined. Below pH 6.5 the exchange rate of calcium in the tooth with calcium in solution decreases. Ca^{45} taken up by the tooth section is not removed by washing, as in the case of absorbed inert material, indicating chemical exchange. Neutron activation of teeth failed to reveal a measurable activation product that could be correlated with age.

15. EXPECTED RESULTS IN FY 1966

1. A summary of mortality rates and age-specific fertility in the free-living Rock Valley rodent population, with a history of the population over a four year period, will be prepared.

2. Investigation of the factors influencing uptake of radioactive calcium by sections of rodent molar teeth will continue, in an effort to develop a method for age estimation of wild rodents.

3. Evaluation of fertility and of blood cell counts in chronically irradiated and control Peromyscus will be accomplsihed to determine if there are effects from the dose rate of one r/day.

4. Monthly census of the rodent populations in the Rock Valley enclosures will continue in an effort to detect effects of low level chronic exposure to ionizing radiation. The spring of 1965 will see the first breeding season after prolonged exposure, and therefore presents the first opportunity to observe effects on fertility.

16. EXPECTED RESULTS IN FY 1967

1. Results of a new method, utilizing chemical exchange of calcium-45 with stable calcium in teeth, for age estimation of wild rodents is expected to be available.

2. Effects, if any, of exposure to one r/day of gamma radiation for prolonged periods should become evident in the rodent population of Rock Valley, Nevada Test Site. The significance of these effects will be evaluated.

3. Utilization of isotopes in the field for evaluation of metabolic rates of wild rodents will be attempted. This method provides the first opportunity to measure energy requirements of free-living animals.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Ecology of the Nevada Test Site

3. AEC Budget Activity No.:
06-05-01

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
J. C. Beatley

8. Project Term:
From: 1961 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	5	6	6
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$
Total	$5\frac{1}{4}$	$6\frac{1}{2}$	$6\frac{1}{2}$

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 44,000	$ 59,400	$ 60,500
(b) Materials & Services	8,200	8,900	10,000
(c) Indirect Expenses* (3%)	33,200	(4%) 43,800	(4%) 44,700
Total	$ 85,400	$ 112,100	$ 115,200

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Survival of winter annuals in the northern Mojave Desert. Submitted to Ecology, January, 1965.

2. Ecological status of introduced species at the Nevada Test Site, with special reference to Bromus rubens L., B. tectorum L., and Salsola kali L. Submitted to Ecology, January, 1965.

3. Post-Sedan two years: Effects on desert vegetation of a nuclear detonation, Nevada Test Site. Submitted to Radiation Botany, January, 1965.

4. (With William H. Rickard) Canopy-coverage survey of the desert shrub vegetation mosaic of the Nevada Test Site. Ecology, 1965. (in press).

5. Effects of radioactive and non-radioactive dust upon Larrea divaricata Cav., Nevada Test Site. Battelle-Northwest Symposium, Health Physics 11 (12). 1965.

6. Ecological status of introduced species at the Nevada Test Site. Bull. Ecol. Soc. Amer. 45: 78. 1964. (abstract).

7. Effects on desert vegetation of a nuclear detonation, Nevada Test Site. Bull. Ecol. Soc. Amer. 45: 80. 1964. (abstract).

8. The vascular flora of the Nevada Test Site, Nye County, Nevada. Amer. Jour. Bot. 51: 687. 1964. (abstract).

9. Ecology of the Nevada Test Site. I. Status of introduced species. UCLA ____. 1965. (in press).

10. Ecology of the Nevada Test Site. II. Survival of winter annuals, 1963-64. UCLA_____. 1965. (in press).

11. Ecology of the Nevada Test Site. III. Effects on shrub vegetation of a nuclear detonation, two years post-sedan. UCLA_____. 1965. (in press).

12. Vascular plants of the U. S. Atomic Energy Commission's Nevada Test Site, Nye County, Nevada (revised and annotated). UCLA___. 1965. (in press).

12. SCOPE OF THE PROJECT

The program encompasses field and laboratory studies necessary for (1) the establishment of biological and environmental-effects baselines for ecosystems in all regions of the Nevada Test Site, and (2) the determination of the effects upon native plants and animals of increased intensities of ionizing radiation from nuclear testing, and effects of changed intensities of other environmental variables associated with test activities.

13. RELATIONSHIP TO OTHER PROJECTS

Ecological studies conducted on other AEC-DBM contracts, especially Brookhaven National Laboratory, Emory University, and Oak Ridge National Laboratory, and desert biological studies in various institutions, have a direct or indirect relationship to the plant, animal, and environmental studies being conducted on this project at the Nevada Test Site.

14. TECHNICAL PROGRESS IN FY 1965

(1) Vegetation studies. Phytosociological and phenological data have continued to be collected at the appropriate seasons on all of the 68 permanent study plots of the network established in 1962. These have included quantitative data for winter annuals, summer annuals, and herbaceous perennials on all plots, and in addition, for the shrubs on the disturbed-site plots of the network; biomass data for all plots; and winter annual survival data on certain of the sites. A photographic record of the vegetation on all plots was obtained. All data are being recorded on IBM punch-cards, and computer programs are being written for their analyses.

Radioisotopes, injected into plants and soils, are being used to study rooting habits and interspecific exchange of minerals. Excavation is employed to substantiate the large mass of data obtained by the use of nuclides. Anatomical studies are underway to obtain an understanding of the clonal nature of desert plants and their adaptation to arid climates and soils.

(2) Animal studies. Consecutive nights of trapping of the small mammals on all plots was conducted, the data recorded on punch-cards, and a program is being written for correlations with plant and environmental measurements.

(3) Environmental measurements. Weekly records of precipitation, maximum and minimum air temperatures, soil moisture, soil temperature, and ionizing radiation have continued to be collected on all of the permanent study sites. Soil moisture studies are collaborative with Dr. H.A. Hawthorne.

Particle-size characterizations and pH determinations of soils from all plots were completed.

(4) Radiation effects studies. Final data for the Larrea radiation effects study were obtained with a third year of germination trials conducted under controlled environmental conditions at the Laboratory. All data are being recorded on IBM punch-cards, and a program is being written for computer analyses which will compare all parameters in the irradiated and non-irradiated populations as expressed during the several seasons for which measurements and experimental data have been obtained.

Vegetational phenomena of the permanent study plots in the Sedan area have been compared at all seasons with the expression of the same phenomena elsewhere in Yucca Flat, and in other Test Site drainage basins. Neither deleterious nor beneficial effects of ionizing radiation have yet been demonstrated in shrub or herbaceous species. Causes of abnormal growth of a winter annual species (Chaenactis stevioides) are being investigated in collaboration with Dr. D.W. Kyhos, Stanford,Univ.

Additional collections of plant materials for chromosome number/nuclear volume studies were made.

(5) NTS Herbarium. Around 700 collections from the Test Site were accessioned. Approximately one-half of these were reviewed by group specialists associated with other herbaria in the country.

15. EXPECTED RESULTS IN FY 1966

Vegetation and small mammal studies, and environmental measurements, will continue to be made, and the data recorded on IBM punch-cards for use in computer correlation analyses programs being written. A limited number of the permanent study sites will be instrumented with automatic recording equipment.

Radiation effects studies will continue in the Sedan area, with the emphasis turning toward experimental studies designed to elucidate certain problems of interpretation of the behavior of Sedan populations. These include the basis for predicting relative radiosensitivities through chromosome number/nuclear volume determinations of the more than 100 species of vascular plants in the Sedan area.

It is anticipated that a Ph.D. Cytologist, with experience in the cytology of desert vascular species, will be added to the staff for a period of two years to conduct these studies.

Collections for the herbarium will continue, with greatest intensity on the Test Site, but also throughout the Las Vegas Bombing and Gunnery Range to the north of the Test Site, and in certain other areas of southern Nevada.

16. EXPECTED RESULTS IN FY 1967

Field data of the kinds collected during the past three years will continue to be collected, and an increasing number of laboratory studies will be initiated in support of the field program. No physical expansion in the field program is anticipated.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Radiation Ecology - Lizard Irradiation

3. AEC Budget Activity No.:
06-05-01

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
F. B. Turner

8. Project Term:
From: 1962 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 3/4	8	8
(b) Other Tech.	1 1/2	0	0
Total	6 1/4	8 **	8

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 52,500	$ 82,500	$ 86,900
(b) Materials & Services	7,800	9,700	11,700
(c) Indirect Expenses*	(4%) 43,400	(5%) 54,900	(5%) 56,000
Total	$ 103,700	$ 147,100 **	$ 154,600

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

** Increases in FY 1966 and FY 1967 are the result of the transfer of all research from Activity 06-05-03.

11. PUBLICATIONS DURING FY 1965

1. A survey of the herpetofauna of the Death Valley area. Great Basin Nat. 23: 119-128. (with Roland Wauer)

2. Aridity and man in the western United States. Ecology 46: (in press)

3. Studies of the lizard, Anolis gundlachi, in a Puerto Rican forest. Bull. Ecol. Soc. Amer. 45: 115. (with C. Gist)

12. SCOPE OF THE PROJECT

The effects of low levels of external irradiation on natural populations of animals are not known. Studies of such effects may be designed in several ways. One approach involves a continuing exposure of all members of a population to the same low level of gamma radiation. Another involves the exposure of members of a population to a gradient of radiation intensity, so that individuals receive varying doses. Either approach yields information of value in understanding the influence of environmental radiation on natural communities, and estimating its possible effects on man.

The Rock Valley study entails the continuous irradiation of natural populations in such a way that all individuals receive approximately equal doses. One objective of this study is to define the effects, if any, of continuous exposure to low levels of gamma radiation on populations of lizards and the arthropods on which these reptiles prey. The Rock Valley study also considers the impact of chronic irradiation on the whole community as an entity, both in terms of its organization and function. Special emphasis is being placed on the comparative energy metabolism of irradiated and non-irradiated communities.

The effects of gamma radiation from a 10,000-curie Cs-137 source on a section of tropical forest is being studied by the Puerto Rico Nuclear Center. Because the most numerous vertebrates in the forest are lizards and frogs, determination of the dispersion, density, and age-distributions of populations of these animals, and how such attributes may be modified by exposure to gamma irradiation is being investigated by our laboratory.

In both of the above studies, it is important to evaluate the dose experience of individuals as precisely as possible. Although free -air doses will be documented in both study areas, these data will not be applicable to the dosages experienced by animals. Therefore, tissue doses to individual organisms will be estimated by the use of small thermoluminescent dosimeters.

In both the Rock Valley and Puerto Rican studies, the primary objective is to seek evidence of modification of population parameters by irradiation, and to correlate any observed changes with dose experience. However, it is known that the incidence of chromosome aberrations in animals is increased by exposure to gamma irradiation. It is proposed to determine ddetails of normal chromosome number and morphology in selected lizard species and compare normal karyotypes with those of individuals irradiated in the course of the field experiments.

13. RELATIONSHIP TO OTHER PROJECTS

The Rock Valley project uses facilities designed by Dr. Norman R. French. Whereas Dr. French is investigating the long-term effects of irradiation on small mammals, the study discussed here pertains to lizards. Dr. Donald Tinkle and his students at Texas Technological College in Lubbock, Texas,

are carrying out experiments on the effects of X-irradiation on lizards (Uta stansburiana). Studies on the effects of X-irradiation of toads are being carried out by Dr. Frank Blair at the University of Texas. Studies of the effects of chronic gamma irradiation on selected arthropods have been conducted at Brookhaven National Laboratory under the direction of Dr. George Woodwell. Dr. Frank Golley and Dr. Ernest E. Provost at the University of Georgia's Savannah River Project are investigating radiation effects on various species of wild mammals. The relationship of the Puerto Rican study to the rainforest project directed by Dr. Howard T. Odum has already been described.

14. TECHNICAL PROGRESS IN FY 1965

(1) Rock Valley

The radiation source, 33,600 curies of Cs^{137}, was activated in Rock Valley during February, 1964. Hence, during FY 1965 plants and animals in the experimental enclosure were exposed to almost continuous gamma irradiation. A great deal of effort was invested in capture and marking of the small but numerous Uta stansburiana. This work has continued throughout the winter, and it is expected that by May, almost every individual of this species in the four areas will be registered. Weight-length relationships of three species of lizards have been evaluated in one area in 1963 and four areas in 1964. The regression equations for Uta are all quite similar, implying neither year-to-year differences nor differences between plots. Equations for the other species are somewhat different, but probably not significantly so. Growth of Uta stansburiana hatched during July, 1964, was followed during the summer and fall of 1964. Males grew more rapidly than females. It is also likely that the lizards in Plot D (unfenced) showed significantly more rapid growth than lizards in the other plots. No other differences appear likely.

Several experiments involving the acute irradiation of Uta stansburiana were carried out. In one experiment the survival of animals exposed to 1300 to 1400 roentgens was as good as that of controls, but the controls exhibited greater increase in body length and body weight. Increase of body length was effectively suppressed in animals exposed to 1500 or more roentgens, but even some of these animals showed modest weight gains during the 30-day period. Another experiment involved the 30-day experience of groups of 5 males and 5 females exposed to doses ranging from 1800 to 3600 roentgens, delivered at 204 r/minute. This experiment suggested a greater radioresistance in females. Whether this is true at all times of the year is not yet known. Such a difference between sexes is not exhibited by juvenile animals. Further experiments along this line are planned.

As part of our study of the metabolism of lizards during the course of a yearly cycle, the body temperatures of Uta stansburiana at different times of day and season were measured in Rock Valley. These measurements, and similar ones for other species and other times of year, will be used in a model of the energy budget of these species in the analysis of the overall metabolism of the Rock Valley communities.

During the spring of 1965, small thermoluminescent dosimeters furnished by E. G. & G. were implanted in a number of Uta stansburiana. The ultimate recovery of these dosimeters, by careful excision, will affort estimates of the absorbed dose experienced by individuals over a period of time. Similar studies with other species of reptiles are envisioned.

Analyses of normal karyotypes of several species of Rock Valley lizards have been made, using testes squashes and white blood cell culture techinques. The latter method is more difficult but seems to yield better preparations. Work along these lines is continuing during the spring of 1965.

Sampling of arthropods was initiated during the early fall of 1964. Traps were modified by the addition of galvanized metal inserts. Progress has been made in determining the most important species in the Rock Valley area, at least as revealed by fall and winter sampling. Experiments in indoor enclosures have been conducted in order to determine the most effective means of marking selected species. Further methods of sampling, especially of shrub species, are being investigated.

(2) Puerto Rican Forest Study

The forest irradiation experiment conducted by the Puerto Rico Nuclear Institute is designed to explore the effect of three months of chronic gamma radiation (from a 10,000 curie Cs^{137} source) on the composition and function of a montane tropical forest. The study area is located in the Luquillo Experimental Forest near the town of El Verde--about 25 miles east of San Juan -- at an elevation of approximately 1500 feet above sea level.

Our efforts have been focused almost exclusively on three species: Anolis gundlachi, A. evermanni, and Eleutherodactylus portoricensis. Since November, 1964, most of the data pertaining to Anolis gundlachi have been analyzed. The repeated sampling of Anolis gundlachi in two 0.7 areas in the Luquillo Experimental Forest indicates that for every attribute so far evaluated, the populations of the two circles are virtually identical. Hence, one can be legitimately used as a control following the irradiation of the experimental area. Quantified measures of numbers of lizards, weight-length relationships, growth, mobility, and other characteristics have been developed, with which may be compared the same parameters following the irradiation.

The density of Anolis gundlachi is estimated at between 350 and 900 per acre, with 500 per acre as a conservative best estimate. The equation for regression of body weight (Y) on body length (X) in Anolis gundlachi is $Y = .003X^2 - .091X + .305$.

Female A. gundlachi attain a size of 47 to 48 mm (snout-vent length) and apparently stop growing. Males may attain 72 to 75 mm in snout-vent length. The sex of most animals less than 50 mm in snout-vent length cannot be ascertained unless subsequent growth records are available. A typical population of A. gundlachi shows two peaks in the size-distribution--one at around 65 mm (adult males) and another at 45 to 46 mm (adult females and younger males). The relative abundance of smaller (younger) individuals is not surely known, because these animals are relatively inconspicuous and are often on the ground where it is difficult to capture them. Both Anolis gundlachi and A. evermanni were captured mainly between about 2 and 5 feet from the ground (means for animals over 50 mm in snout-vent length were 3.88 ft and 3.78 ft respectively). Depending on the species and the size of individuals, from about 7 to 16% of the animals captured were on the ground. Perches used by the two species were usually 1 to 3 inches in diameter.

Anolis gundlachi is a fairly sedentary species. Average movements were around 6 or 7 m. Animals above 50 mm in snout-vent length (all males) were shown to be somewhat more vagile (mean of 240 movements = 7.22 m) than smaller males and females (mean of 240 movements = 6.00 m). Time of year did not influence vagility significantly.

Ideally, the possible influences of the irradiation need to be evaluated in terms of absorbed dose. Free-air doses are to be documented in detail by McCormick. However, it is expected that absorbed doses will be appreciably (and possibly considerably) lower than free-air doses. We hope to estimate the approximate magniture and variability of absorbed doses in various individuals of the populations by the use of implanted thermoluminescent dosimeters. The dosimeters used were prepared by Edgerton, Germeshausen & Grier, and consisted of a glass cylinder 6.0 mm long and 0.9 in diameter containing lithium fluoride powder. Dosimeters recovered after the irradiation will be read by E. G. & G., cleared and then calibrated against known dosages. The original reading will then be converted to roentgens of abosrbed dose. Read-out may range between 1 to 100,000 roentgens. These dosimeters show very little over-response to low-energy gamma radiations (i.e., the response is very close to energy-independent).

Between December 14 and 21, the dosimeters were implanted beneath the skin of lizards and tree frogs in the experimental area. Most of the 172 animals so treated were captured and released between 10 and 30 m. from the source. However, some were treated between 30 and 45 m.

15. EXPECTED RESULTS IN FY 1966

Sampling of lizards in four 20-acre areas in Rock Valley will be continued. If a new, more powerful source is installed the sampling schedule will be modified accordingly. Special efforts will continue to be focused on Uta stansburiana. Adult lizards of several species will be collected in Rock Valley in order to determine the ovarian cycle, and the relationship of body size and fertility.

Internal dosimetry with small thermoluminescent dosimeters will be continued in Rock Valley. Such dosimeters are presently obtained from E. G. & G. The company also reads dosimeters recovered from the field. An effort will be made to estimate the frequency distributions of absorbed doses in the experimental Rock Valley populations. Studies of the effects of acute gamma irradiation on lizards will continue, with emphasis on how dose responses are modified by sex, age, and time of season.

Studies of the metabolism of the important species will be continued, as well as measurements of the normal thermal regimes of these species. Methods of estimating the body temperatures of active animals have already been developed, but the problem of determining the temperatures of inactive animals (at night or during hibernation) remains. Small activated tantalum wires will be injected into selected individuals, so as to be able to locate these animals underground by using a scintillometer. Soil temperatures, which are reliable indices of body temperatures of submerged lizards, will be automatically recorded at 6 depths ranging from 1 inch to 12 inches in a number of selected localities in Rock Valley. These data, in conjunction with known depth distributions of inactive lizards, will be used to derive the temperature experiences of animals at night and during the winter. These data will be integrated with similar observations on other animal species as an estimate of the energy metabolism of the Rock Valley areas.

In this conjunction, a program involving bomb calorimetry will be begun. These investigations will contribute necessary data on the energy dynamics of the Rock Valley areas.

Investigations of normal chromosome number and morphology of lizards will continue. Blood samples cultured from animals taken from the continuously

irradiated plot will be used to determine whether gross chromosomal aberrations are accumulating in the irradiated populations.

Sampling of arthropods will continue. It is expected to add an entomologist to the Rock Valley group during FY 1966. This individual will take over the responsibility for investigations of arthropod species. Determination of the most important species in the function of the areas will continue. Sampling will be expanded to include techniques other than traps. Marking studies involving certain species of tenebrionid beetles will be initiated in an attempt to estimate the absolute numbers of these important species.

Field work in Puerto Rico will be concluded. Analysis of data acquired during the post-irradiation sampling will begin.

Efforts directed towards more efficient processing and analysis of data will be continued, with emphasis on the use of existing programs in the Biomedical Library and the development of special programs as required.

16. EXPECTED RESULTS IN FY 1967

The program in 1967 will be almost exclusively concentrated in Rock Valley, with emphasis on populations of reptiles and arthropods. The same methods outlined before will be used, together with improvement and modifications developed during FY 1966. The general areas of endeavor will be as before:

1. Continued sampling of lizards and arthropods
2. Analysis of size-specific fertility in lizards
3. Internal dosimetry
4. Bomb calorimetry involving important Rock Valley species
5. Metabolism of lizards and its relationship to the annual temperature regime
6. Acute irradiation experiments with lizards
7. Continued improvements in data reduction by mechanical means

The development of power reactors at San Onofre and Malibu may offer occasion for studies of radioactive isotopes in coastal waters. Background samples from the vicinity of the San Onofre reactor site are being collected now by the Southern California Edison Company. These samples include kelp, non-migratory fish, lobsters, and various molluscs, and will be the basis for evaluating potential influences of the reactor wastes. If levels in marine organisms are high enough to enable routine radiochemical analyses, it is our intention to explore the possibility of examining some simple food-chain relationship involving an economically important herbivorous species and its food supply. Theidea will be to develop a mathematical model capable of predicting levels of one or a group of radioisotopes in a marine primary consumer as a function of levels in the water and in the source of food. Such a model would presumably be capable of predicting the outcome of situations in which the amounts of radioisotopes in littoral waters were, for some reason, well above safe levels.

Opportunities for studies in Central America may arise if the construction of a sea level canal by means of nuclear explosives is approved. At this time we do not propose active participation, but merely to follow developments as they take place.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
   University of California, Los Angeles

   Contract No.: AT(04-1)GEN-12

2. **Project Title:**
   Radiation Ecology - Plant Irradiation

3. **AEC Budget Activity No.:**
   06-05-01

4. **Date Prepared:** April 1965

5. **Method of Reporting:**
   Publications, UCLA Reports
   Semi-annual and Final Reports

6. **Working Location:**
   UCLA and Nevada Test Site

7. **Person in Charge:**
   W. E. Martin

8. **Project Term:**
   From: 1962 **To:** Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 3/4	7	7
(b) Other Technical	1/2	0	0
Total	5 1/4	7	7

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 36,300	$ 70,400	$ 72,600
(b) Materials & Services	4,800	7,900	8,600
(c) Indirect Expenses*	(3%) 33,200	(5%) 54,900	(4%) 44,700
Total	$ 74,300	$133,200 **	$125,900

* **Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.**

** Increases in FY 1966 and FY 1967 are the result of the transfer of all research from Activity 06-05-03.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

The purpose of this project is to study the effects of nuclear detonations and of ionizing radiation, both acute and chronic, on natural plant populations and plant communities. The studies now in progress and those planned for the future are intended to provide information concerning: (1) the relative importance of throwout deposition, air blast, base surge dust deposition, and ionizing radiation in predicting the long term, close-in effects of nuclear excavation on vegetation; (2) the effects of high-level, acute and low-level, chronic radiation on the: (a) growth, (b) reproduction, and (c) productivity of plant populations and communities.

The effects of throwout deposition, air blast, base surge dust deposition, and ionizing radiation on vegetation within a radius of 10,000 ft. from Sedan GZ (ground zero) have been observed since 1962. In areas less than 5,000 ft. from GZ, the effects of throwout deposition and air blast were so intense that the effects, if any, of ionizing radiation were obscured. In areas between 5,000 and 10,000 ft. from GZ, cumulative gamma exposure doses were relatively low (less than 4000 r), and the effects on plants were generally minor and/or obscured by the simultaneous effects of heavy base surge dust deposits.

To study the combined and separate effects of ionizing radiation and dust deposits, plant populations about 12,000 ft. upwind from Sedan GZ were exposed to gamma irradiation from a 1200 curie Co^{60} source. Some of the irradiated plants were covered with dust and some were not. Estimates of these treatment effects will be based on comparisons between irradiated and nonirradiated populations in the same locality. Plans are being made to perform a series of similar experiments to study the effects of high-level gamma radiation in combination with other environmental treatments on plant populations and communities.

The facilities established for Dr. N.R. French's study of radiation effects on small mammal populations (see UCLA Rpt. 12-532) are being used to study the effects of chronic, low-level gamma radiation (2 to 4 r/day) on natural plant populations and communities. Comparisons between the irradiated plot (20 acres) and three nonirradiated plots are based on: (1) measure of environmental factors such as radiation doses to plants, air and soil temperatures, rainfall, and soil moisture, (2) estimates of population parameters such as density, frequency, and dominance, and (3) measurements of morphological parameters such as shoot length and the numbers of nodes, leaves, buds, flowers, and fruits per shoot. Most of the environmental and morphological measurements are made each month, and these are used to establish phenological relationships, growth rates, and indexes to reproductive success. These and other data will be used, eventually, to compare the productivity of the irradiated plot with that of nonirradiated plots.

The results of studies in the Sedan area should be useful in the planning of future Plowshare events and in attempting to predict the long-term, close-in effects of nuclear excavation on vegetation. Studies concerning the effects of acute, high-level and chronic, low-level radiation should be useful in attempting to define the tolerances of plant populations and communities to ionizing radiation in combination with other environmental

stresses. Of particular interest are the effects of sublethal radiation levels which might be insignificant in relation to the survival of individual plants but deleterious to the reproduction and maintenance of plant populations and/or the development and productivity of plant communities.

13. RELATIONSHIP TO OTHER PROJECTS

Dr. J. C. Beatley, of this Laboratory, is studying the effects of Project Sedan on vegetation in two permanent plots located 5,000 and 10,000 ft. from Sedan GZ. Dr. F. B. Turner, also of this Laboratory, has made a study of lizard populations; and Dr. Clive Jorgansen, of Brigham Young University, is making a study of small mammal populations in the Sedan area. Studies of vegetation in other target areas at the Nevada Test Site have been made by Drs. L.M. Shields and P.V. Wells of Highlands University. Similar studies have been made at the Pacific Proving Grounds by Dr. R.F. Palumbo of the University of Washington.

The study of chronic, low-level radiation effects on plants is closely related to similar studies of invertebrate, lizard, and small mammal populations being made in the same area by Drs. F.B. Turner and N.R. French of this Laboratory. Studies of high-level radiation effects on natural plant populations and plant communities are being made by Dr. G.M. Woodwell at the Brookhaven National Laboratory, by Dr. R.B. Platt at Emory University, and by Dr. H.T. Odum at the Puerto Rico Nuclear Institute.

14. TECHNICAL PROGRESS IN FY 1965

By the summer of 1964, Salsola kali was generally more abundant in the throwout zone (650 - 2000 ft. from Sedan GZ) and in the blast zone (2000 - 5000 ft. from GZ) than it had been before the detonation in 1962. Other herbaceous species were less abundant in 1963 and 1964 than they had been in 1962, but this has been attributed to a lack of rainfall during the autumn and winter months of 1962 and 1963 - not to the Sedan test. Between 1963 and 1964, the number of living shrubs in the throwout and blast zones increased by 5 to 10%. There was an increase in the abundance of Salsola kali throughout the base surge zone (5000-10,000 ft. from GZ), but there was no new evidence of damage to shrubs other than Larrea divaricata which were partially or completely defoliated in 1962, produced some new foliage in 1963, and continued to produce new foliage in 1964.

In August 1964, a field experiment was performed to study the separate and combined effects of dust and radiation on Larrea divaricata and other shrub species. Two plants (Larrea) which were exposed to about 25.000 r began to loose their leaves about two months after exposure, and the plant exposed to both dust and radiation was more severely damaged than the one exposed only to radiation. Observations of the growth and reproduction of irradiated plants and nonirradiated plants in the same locality will continue during the summer of 1965.

Environmental measurements in the Rock Valley area have shown only minor differences between the radiation field and the control plot. Monthly measurements of various morphological parameters have shown that shoot growth, and leaf production was essentially completed by May 1964. The shoot growth of some species (e.g., Larrea divaricata) was significantly greater in the control plot than in the irradiated plot. On the other hand, Krameria parvifolia shoots were significantly longer in the irradiation field than in the control plot. For other species (e.g. Lycium

andersonii), there was no significant difference between the average lengths of shoots in the radiation field and control plot. The only growth noted after May was in response to a relatively heavy rain in July. Between July and August, there was an increase in the length of Larrea shoots in both the irradiated and the nonirradiated plots, but the amount of increase was the same in both plots. Lycium andersonii had no foliage in July but produced a new crop of leaves in August. The percentage of shoots producing leaves was significantly higher in the control plot than in the irradiated plot. Larrea and Krameria both responded to the rain in July by producing lateral shoots. The number of lateral shoots produced (between July and September) by Larrea was higher in the control plot while the number of lateral shoots produced by Krameria was higher in the radiation field, but there was no significant difference between plots of the rate of lateral shoot growth of either species.

The most interesting results obtained in 1964 are related to the flowering of Larrea divaricata and Ephedra nevadensis. Both of these species are characterized by large cell nuclei (over 450 cubic microns), and the evidence obtained in May indicated that the flowering of both species was inhibited in the radiation field. In 1965 we will inaugurate an expanded study of flowering and reproductive success in the irradiated and non-irradiated plots in Rock Valley and perhaps in several other locations.

Because of the high degree of variability within plots, i.e., among the individual plants in each plot, we hesitate to attribute the observed differences - even though they are statistically significant - to the effects of low-level gamma radiation. The accumulation of data over a period of years, a minimum of three years, may establish trends and help to determine the source or sources of observed variability within and differences between the radiation field and the control plots.

15. EXPECTED RESULTS IN FY 1966

Observations of plant succession in the Sedan area will be continued but confined to several permanent plots in which more detailed observations can be made.

Studies of the effects of high-level radiation and other environmental treatments on plant populations and communities will be expanded. Plans are being made in collaboration with Dr. R.B. Platt of Emory University to conduct a series of field experiments utilizing a 1200 curie Co^{60} source which can be moved from one place to another. The results of these experiments should help to establish the relative sensitivities of different species and of different biological functions, especially growth and reproduction, to ionizing radiation. Information will also be obtained concerning the effects of other environmental stresses on the growth and reproduction of irradiated plants.

Studies will also be made to determine whether or not desert shrubs can be propagated from branch cuttings. If this method of propagation is feasible, it may be used to study the responses of plants which are first exposed to gamma radiation under field conditions and then transferred to a controlled environment and subjected to other experimental treatments.

Measurements of environmental and morphological parameters in the Rock Valley area will be continued at about the same level as in 1965 except that the production of flowers, fruits, and seeds by plants in and out of the radiation field will be studied in greater detail. Seeds produced during the summer of 1965 will be collected from both the irradiated and the non-

irradiated plots and tested to determine their viability and germinability.

Preliminary studies related to productivity will also be undertaken during FY 1966. Our first objective will be to obtain a monthly inventory of chlorophyll in the irradiated and nonirradiated parts of the Rock Valley study area. Plant samples will be collected at the same time other observations are being made; and these will be used to determine the cholorphyll content of different plants in terms of chlorophyll per unit dry weight and per unit of surface area.

16. EXPECTED RESULTS IN FY 1967

By the end of FY 1966, most of the studies in the Sedan area should be completed. In FY 1967, it is anticipated that studies of the effects of acute, high-level and chronic, low-level radiation on plant populations and communities will continue at about the same level as in FY 1966.

Plans are now being made in collaboration with Dr. W.A. Rhoads of this Laboratory to undertake a series of field studies related to the productivity of desert plant communities. The new studies involved will be concerned primarily with the measurement of plant photosynthesis and respiration under field conditions and in the laboratory. These data, combined with the chlorophyll inventory mentioned above and data concerning the biomass and energy content of various communities, will enable us to estimate and compare the efficiencies of different plant communities in fixing energy and making it available, in the form of food, to support higher trophic levels.

It is not expected that the exposure of plants to sublethal levels of ionizing radiation will impair their ability to carry on photosynthesis, but it could, by other means, decrease the productivity of a plant community. If the growth of photosynthetic tissue is inhibited by radiation, the total amount of chlorophyll and of photosynthate produced by a population or community would be reduced. If radiation inhibits the production of flowers, fruits and seeds, the amount of food available for higher trophic levels would be reduced. Thus by minor alterations in the growth and reproductive patterns of plant populations the productivity of a plant community would be reduced by a level of chronic radiation which is not lethal to any individual plant in the community.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Chemical Problems - Ecology

3. AEC Budget Activity No.:
06-05-01

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
R. A. Wood (Acting)

8. Project Term:
From: 1956 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 ½	10 ½	11
(b) Other Tech.	½	0	0
Total	5	10 ½ **	11

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 42,800	$ 83,600	$ 90,200
(b) Materials & Services	11,600	27,400	41,400
(c) Indirect Expenses* (3%)	31,100 (5%)	54,900 (5%)	56,000
Total	$ 85,500	$ 165,900 **	$ 187,600

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

** Increases in FY 1966 and FY 1967 are the result of the transfer of all research from Activity 06-05-03.

11. PUBLICATIONS DURING FY 1965

Will be found in the programs of the various sections supported by this Section.

12. SCOPE OF THE PROJECT

The Chemical Problems Section has as its basic purpose the support of the program objectives of the Environmental Radiation Division in its requirements for radiochemistry. The individual needs of the various sections are reflected in their program objectives. The secondary objective of this section is the search for and development of new analytical procedures, particularly adaptable to the sequential separation schemes used in low-activity determinations. The Environmental Decay Section, in particular, has required large numbers of analyses for Sr^{90}, Ce^{144}, Cs^{137}, Zr^{95}, Ra^{226}, and the stable elements, Sr, K, P and Ca. While in the past our primary effort has been on Sr^{90}, Ce^{144}, and Cs^{137}, we are presently equipped and staffed to perform analyses for other fission products and a number of stable elements by standard, or recently-developed radiochemical and gamma spectrometric methods, where applicable.

Other services rendered by this section are the preparation and standardization of isotopic solutions and preparation and calibration of counting standards.

Several continuing programs are being carried out as joint projects with investigators in this Division and other Divisions. Publications reflecting the work of this Section will be found in the various divisional program presentations.

13. RELATIONSHIP TO OTHER PROJECTS

Analytical work of a similar nature is carried on in the following organizations. Similar research is under way in many of them.

Analytical Branch, Health and Safety Laboratory, New York Operations Office.

Applied Fisheries Laboratory, University of Washington, Seattle, Washington.

Lamont Geological Laboratory, Columbia University, New York City.

Ecological Research Project, Oak Ridge National Laboratory, Oak Ridge, Tenn.

U. S. Naval Radiological Defense Laboratory, San Francisco, California.

Lawrence Radiation Laboratory, Livermore, California.

The relationship of this Section to other Sections of the Environmental Radiation Division is indicated in the "Scope of the Project".

14. TECHNICAL PROGRESS IN FY 1965

During the first eight months of fiscal year 1965, content of Sr^{90}, $CePr^{144}$, Ca^{137}, Zr^{95}, Ra^{226}, the stable elements of Sr, Ca, Ba, K and P have been analyzed in a large number of milk, plant, water and soil samples. A limited number of air filters, and muscle and bone samples were also analyzed for Sr^{90}, $CePr^{144}$, Cs^{137} and Sb^{125}. Approximately 8000 determinations were completed during this period of time.

This analytical work also required much time and effort in preparation of counting standards to allow quantitative interpretation of data obtained from gamma spectrometry. Gamma spectrometry achieved full operation in support of Division program objectives. A very considerable amount of work was completed on several sequential analytical procedures needed for the analysis of neutron-induced radionuclides in various kinds of soils in a continuing program of the Soil Factors Section. Final manuscripts on these procedures are in process. Further details of the samples processed follow:

Environmental Decay studies, Dairy Farm Project: Milk, soil, muscle tissues and plants were analyzed for Sr^{90}, Ce^{144}, Cs^{137}, Ra^{226}, Zr^{95}, Sb^{125}, and the stable elements Sr, Ca, Ba, P and K. It is expected that samples from this section will constitute 95% of all samples analyzed for the next four months of this fiscal year. A tenfold increase in the total number of determinations is expected during the next two fiscal years due to the need for more information about the stable elements.

15. EXPECTED RESULTS FOR FY 1966

The determination of radionuclides of very low activities will be continued at approximately the same level for the next two fiscal years, with a probable increase in effort directed to other fission and induced radionuclides such as W^{185}, Sb^{125} and Mn^{54}. This section expects to perform at least 10,000 determinations during the coming fiscal year. With the increased capability for quantitative spectrometry, we anticipate an expansion in research in the gamma-emitting, fission and induced products in various environmental research projects.

16. ANTICIPATED PROBLEMS FOR FY 1967

No major changes in direction are indicated, although procedure-development for analysis of neutron-induced isotopes is expected to require much more time than in the past. Samples of very low or near-background activity will continue to dominate the analytical work of this section.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
Chemical Problems - Fallout

3. **AEC Budget Activity No.:**
06-05-03

4. **Date Prepared:**
April - 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
R. A. Wood (Acting)

8. **Project Term:**
From: 1956 **To:** 1965

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 1/2	-0-	-0-
(b) Other Tech.	1 1/4	-0-	-0-
Total	5 3/4	-0-	-0-

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 36,000	$ -0-	$ -0-
(b) Materials & Services	7,900	-0-	-0-
(c) Indirect Expenses* (3%)	30,700	-0-	-0-
Total	$ 74,600	$ -0- **	$ -0-

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

** Transferred to 06-05-01 category in FY 1966 and FY 1967.

11. PUBLICATIONS DURING FY 1965

Publications reflecting the work of this section will be found under Environmental Assessments-Plant Studies, and under Nuclear Events-Biological Studies.

12. SCOPE OF THE PROJECT

The Chemical Problems Section was supported by radiochemical analyses and by gamma spectrometry, the work done under three Sections funded by the Fallout Studies Branch. Traditionally, this has meant the sizing and classification of fallout particles as well as a physico-chemical analysis. This has required the processing of many materials in order to recover fallout materials from extraneous materials for field studies, either from local fallout in the vicinity of the Nevada Test Site, or from materials contaminated by world-wide fallout. The scope of the project is identical in many ways to that covered in Chemical Problems (Ecology) under Budget Activity 06-05-01, and the Section's function as one laboratory, except under the contingency of field programs when this Section has, in the past, assembled temporary laboratories at the Nevada Test Site.

13. RELATIONSHIP TO OTHER PROJECTS

Analytical and research work is carried on in many of the following organizations, and similar research is under way in many of them.

Hazleton Nuclear Science Corporation, Palo Alto, California
Battelle Northwest, Richland, Washington
Applied Fisheries Laboratory, University of Washington, Seattle
LamontGeological Laboratory, Columbia University, New York City
Ecological Research Project, Oak Ridge National Laboratories
U.S. Naval Radiological Defense Laboratory, San Francisco, Calif.
Lawrence Radiation Laboratory, Livermore, California

The work of this Section is an integral part of the other Sections of the Environmental Radiation Division.

14. TECHNICAL PROGRESS IN FY 1965

This Section continued radiochemical analyses and processing of samples in a follow-up of the above-ground nuclear weapons testing of the summer of 1962. This work entailed analyses for Sr^{89} and Sr^{90}, Ce^{144} and Cs^{137} on samples of rain water, and local dust and debris collected by the Environmental Assessment Section during summers of 1963 and 1964. Several hundred radiochemical analyses were made.

In support of the environmental studies of a post-Sedan project, muscle and plant samples collected during the Sedan Operation were analyzed for Sr^{89}, Sr^{90}, Cs^{137} and Ce^{144}. Selected samples were also analyzed for other fission products.

15. EXPECTED RESULTS IN FY 1966 AND FY 1967

The fallout-support analysis work has been carried over to the programs under Fresh Water and Terrestrial Ecology. Further work in this section is not anticipated.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Nuclear Events - Biological Studies

3. AEC Budget Activity No.:
06-05-03

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Frederick B. Turner

8. Project Term:
From: 1962 To: 1965

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	1 1/2	-0-	-0-
(b) Other Tech.	1/2	-0-	-0-
Total	1 1/2	-0-	-0-

10. Costs		FY 1965	FY 1966	FY 1967
(a) Direct Salaries		$ 17,100	$ -0-	$ -0-
(b) Materials & Services		2,400	-0-	-0-
(c) Indirect Expenses*	(17%)	10,200	-0-	-0-
Total		$ 29,700	$ -0- **	$ -0-

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

** Transferred to 06-05-01 category in FY 1966 and FY 1967.

11. PUBLICATIONS DURING FY 1965

1. Food-chain relationships of Iodine -131 following the Sedan test of July, 1962. U.S. Atomic Energy Commission Report PNE-236F, 58 pp. (with William E. Martin).

2. Food-chain relationships of radiostrontium in the Sedan fallout field. U.S. Atomic Energy Commission Report PNE-237F, 56 pp. (with W.E. Martin).

3. The uptake of fallout radioisotopes by mammals and a stochastic simulation of the process. Proceedings of the Second AEC Fallout Conference, TID-7678.

12. SCOPE OF THE PROJECT

The accumulation and cycling of radioactive materials in consumer populations, and the doses to which individuals of these populations are exposed, is an important aspect of health physics. Studies of cycling have been augmented by the use of mathematical models which define the time-specific relationships of radioisotopes in various compartments of the environment.

13. RELATIONSHIP TO OTHER PROJECTS

Work related to one or another portions of the above is being carried out in other sections of the Environmental Radiation Division of the Laboratory of Nuclear Medicine and Radiation Biology, and at the:

Oak Ridge National Laboratory, Oak Ridge, Tennessee
Savannah River Project, University of Georgia, Athens, Georgia
University of Utah, Salt Lake City, Utah
University of Minnesota, Minneapolis, Minnesota
University of Colorado, Boulder, Colorado
University of Washington, Seattle, Washington
Brigham Young University, Provo, Utah
University of Nevada, Reno, Nevada
Puerto Rico Nuclear Institute, San Juan, Puerto Rico
Batelle-Northwest, Richland, Washington
National Reactor Testing Station, Arco, Idaho

14. TECHNICAL PROGRESS IN FY 1965

With the publication of the reports listed above, and with the cessation of above-ground nuclear events of major biological interest, investigations of the biological aspects of nuclear detonations have been concluded. The application of stochastic theory to problems of fallout uptake and metabolism was an important conceptual advance. Some of the salient points developed at the Second Fallout Conference follow.

A deterministic model, designed to predict time-specific levels of I^{131} in the thyroids of herbivores as a function of I^{131} on vegetation, was revised as a probabilistic simulation of the experience of a consumer in a fallout field. The stochastic model was used to generate synthetic "populations" of up to 1000 individuals. The frequency distributions of thyroidal I^{131} in 24 of these hypothetical populations were analyzed in terms of the recommended assumption of the Federal Radiation Council, viz. that the majority of individuals in a population does not vary from the average by a factor greater than three. In only two of the distributions did more than 2% of the population exceed three times the mean.

The frequency distributions predicted by the model were all skewed to the high side, and approximated lognormal distributions. A number of frequency distributions of radioisotopes recorded in the literature were reviewed, and Chi-squared tests indicated that most of them were not normal. All of the non-Gaussian distributions were skewed to the high side, and some of them were apparently lognormal. It was concluded that the general form of the synthetic distributions produced by the model was in agreement with most past observations. It was further suggested that such asymmetrical distributions may be more likely than normal ones. The implications of this possibility, in terms of health physics, was discussed.

Consideration was given to the possible relationship between the frequency distribution of a radioisotope in diets and the frequency distribution of the substance in the tissues of animals consuming these diets.

15. EXPECTED RESULTS IN FY 1966

Work in these areas have been combined with Category 06-05-01.

16. EXPECTED RESULTS IN FY 1967

Same as above.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Environmental Assessments - Plant Studies

3. AEC Budget Activity No.:
06-05-03

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
William E. Martin

8. Project Term:
From: 1963 To: 1965

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2 1/4	-0-	-0-
(b) Other Tech.	1/4	-0-	-0-
Total	2 1/2	-0-	-0-

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 27,700	$ -0-	$ -0-
(b) Materials & Services	2,400	-0-	-0-
(c) Indirect Expenses* (2%)	20,400	-0-	-0-
Total	$ 50,500	$ -0- **	$ -0-

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

** Transferred to 06-05-01 category in FY 1966 and FY 1967.

11. PUBLICATIONS DURING FY 1965

1. Radioecology and the study of environmental radiation. Bull. Torrey Bot. Club 91: 283-323.
2. Losses of Sr^{90}, Sr^{89}, and I^{131} from fallout-contaminated plants. Rad. Bot. 4:275-284.
3. Food-chain relationships of I^{131} following the Sedan test of July 1962. PNE-236F, 58 pp. (with F. B. Turner).

12. SCOPE OF THE PROJECT

The purpose of this project is to investigate: (1) the role of plants in the trophic-dynamics (food-chain relationships) of radionuclides released to the environment by nuclear detonations or reactors and (2) the quantitative relationships of radionuclide concentrations on fallout contaminated plants and in the tissues of herbivores.

Studies have been made which involved the collection of plant samples and rabbits from areas contaminated by radioactive reactor effluents (Kiwi B) or by radioactive fallout from nuclear detonations (Projects Sedan and Small Boy). Samples collected before and at various times after environmental contamination were analyzed to determine their radionuclide contents at the times of collection. These radiochemical data have been used to estimate (1) the fraction of fallout intercepted by plants, (2) initial concentrations of radionuclides on plants in relation to H + 24 hr gamma dose rates, (3) rates of radionuclide loss from contaminated plants, and (4) rates of uptake and loss or radionuclides by herbivores. These parameters have been used to formulate deterministic and stochastic models representing the time-specific interrelations of radionuclide concentrations on plants and in the tissues of herbivores.

The results of these studies are useful in helping to estimate the biological consequences of environmental contamination by reactor effluents and fallout and in helping to define the parameters and mechanisms of food-chain kinetics.

13. RELATIONSHIP TO OTHER PROJECTS

Most of the research projects of the Environmental Radiation Division of this Laboratory are directly or indirectly concerned with some aspect of the environmental and biological cycling of radionuclides. The Division of Biology and Medicine, AEC, supports a variety of offsite and onsite research projects which deal partly or primarily with radionuclide cycling. Offsite projects are described in TID-13358. The major onsite program in radiation ecology, the Oak Ridge program, is described in TID-16890.

14. TECHNICAL PROGRESS IN FY 1965

A paper now in press "Early food-chain Kinetics of radionuclides following close-in fallout from a single nuclear detonation" (in TID-7701, 1965) was written to summarize the studies related to Project Sedan. This paper describes the theoretical inter-relation of gamma dose rates, fallout deposition rates, and fallout interception by plants. It describes the parameters involved in food-chain kinetics and presents formulations of deterministic models to represent the observed relationship between initial concentrations of Sr^{89} and I^{131} on fallout-contaminated plants and subsequent

concentrations of Sr^{89} in rabbit-bone ash or of I^{131} in rabbit thyroids. This paper also presents deterministic models to represent the transfer of Sr^{89} and I^{131} from pasture plants to cow's milk, to human tissues, and to estimate the radiation doses delivered to human tissues.

A detailed report (PNE-237F) on the research concerning the food-chain kinetics of Sr^{89} and Sr^{90} has been completed and submitted for publication. A detailed report (PNE-238F) on the research concerning fallout interception and retention by plants in the Sedan and Small Boy fallout fields is in preparation and scheduled for completion before the end of FY 1965. A shorter paper concerning the interception and retention of fallout by desert shrubs has been prepared for the Battelle-Northwest Symposium on "Radiation and Terrestrial Ecosystems".

15. EXPECTED RESULTS IN FY 1966 and FY 1967

It is expected that all the significant results of studies related to Brojects Sedan and Small Boy will have been reported before the end of FY 1966. New or unfinished activities in these categories have been transferred to Budget Activity 06-05-01. (See "Radiation Ecology-Plant Irradiation Studies").

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Physical & Radiological Characteristis of Fallout

3. AEC Budget Activity No.: 06-05-03

4. Date Prepared: April - 1965

5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports

6. Working Location: UCLA

7. Person in Charge: K. H. Larson

8. Project Term: From: 1957 To: 1965

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	-0-	-0-
(b) Other Tech.	$\frac{1}{4}$	-0-	-0-
Total	$2\frac{1}{4}$	-0-	-0-

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 28,600	$ -0-	$ -0-
(b) Materials & Services	1,400	-0-	-0-
(c) Indirect Expenses* (2%)	20,400	-0-	-0-
Total	$ 50,400	$ -0-	$ -0-

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None

12. SCOPE OF THE PROJECT

This project is concerned with comprehensive review, evaluation, integration and publication of the work of this Laboratory during the years from 1957-1964, which is related to the distribution, characterization and biological significance of radioactive fission products released from nuclear detonations in the continental United States. This endeavour is important because the data to be evaluated comprises the most comprehensive, and in most instances, the only information relating to environmental contamination with radioactive fallout in the areas 10-250 miles from ground zero. This evaluation is particularly important at this time, since considerable effort is being expended by several organizations (e.g. Stanford Research Institute, AEC's DBM and TAB) in an attempt to predict the consequences of peacetime (e.g. "Plowshare") activity and military uses of nuclear devices. Because the possibility of securing new information on problems of close-in fallout from atmospheric nuclear tests appears to be very remote as a result of the partial nuclear test ban, predictions must be based on extrapolations from existing data much of which has been published without interpretation and in fragmentary or summary form and frequently without an evaluation of the accuracy of the data and without a clear statement of the adequacy and reliability of the methods used and the errors inherent in the studies.

The intention is to interpret and report significant data which we have accumulated and to evaluate realistically the adequacy of the procedures employed and the validity of the conclusions. It is our belief that such an endeavour will be of considerable value in planning for peaceful uses of nuclear energy and as necessary information in the event of nuclear war.

13. RELATIONSHIP TO OTHER PROJECTS

Similar work, in some of its phases, is being performed at the following organizations or institutions:

Plowshare Group, Lawrence Radiation Laboratory
Evaluations Division, U. S. Army Nuclear Defense Laboratory, Edgewood Arsenal, Maryland
Atmospheric Radioactivity and Fallout and Improvements in Atmospheric Tracer Technology- Hanford Works Laboratory No. AT(45-1)-1350
Radiological and Health Physics and Instrumentation; Aersol Studies ORNL-No. W-7405-Eng 2.6
The Formation, Distribution and Characteristics of Radioactive Fallout USNRDL (Freiling)
Development and Evaluation of Methods and Procedures Relating to Environmental Monitoring. NYO-HASL
Study of Post-Attack Environment Resulting from Thermonuclear War, Rand Corp. (AT(04-3) 414-3
The Biological and Environmental Groups, NMRB/UCLA

14. TECHNICAL PROGRESS IN FY 1965

Work is in progress on three final reports on fallout studies done on the following events at NTS:

Shot Sedan (1962) Report No. PNE-225

Shot Danny Boy (1962)	Report No. WT-1818
Plumbbob Test Series (1957-61)	Report No. WT-1488

Special effort is being made to determine the reliability of the data obtained by the particular methods and field equipment used in these studies. This evaluation should allow recommendations as to additional studies and their methods which may provide better data on fallout phenomenology and the characterization and distribution of fallout in possible future nuclear experiments that may release radioactive debris into atmosphere.

15. EXPECTED RESULTS IN FY 1966

The effort on this project will be terminated by June 30, 1965 at this Laboratory. The Senior Investigator is terminating his employment with this Contract and has accepted a position with the Atmospheric Physics Group, Pacific Northwest Laboratory, Richland, Washington.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Excited States of Molecules in Radiation Biology

3. AEC Budget Activity No.:	4. Date Prepared:
06-06-01	April - 1965
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: Richard L. Lehman	8. Project Term: From: 1965 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	1 1/4	3	3
(b) Other Tech.	0	0	0
Total	1 1/4	3	3

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 15,200	$ 24,200	$ 25,300
(b) Materials & Services	700	2,400	3,400
(c) Indirect Expenses*	(1%) 10,200	(2%) 21,900	(2%) 22,400
Total	$ 26,100	$ 48,500	$ 51,100

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

Preliminary work on the project has been in progress for only a short time, consequently there has been no publication.

12. SCOPE OF THE PROJECT

This is an investigation of excited states of molecules in relation to radiation biology. The purpose of the project is to study, by the use of difference spectrophotometry, the ability of various ions and simple molecules to protect biochemicals in dilute solution from radiolysis. The program will include laboratory investigations of the mechanism of electronic deexcitation of primary excited species in irradiated solutions. Also there will be laboratory investigations of the nature of the ultraviolet and especially vacuum ultraviolet charge transfer bands of enzymes, coenzymes and other biochemicals in solution. These bands arise from a transition in which an electron is dissociated from a cation and becomes associated with adjacent bound water molecules. In order to establish that a particular band is of this type one must put the chemical in different solvents, and in each case, search for a shift in the location of the absorption peak.

There will also be *invivo* studies of the efficiency of the protective chemicals in the bread mold *Neurospora*.

13. RELATIONSHIP TO OTHER PROJECTS

Work in other laboratories that has proved useful in our thinking and planning is by R.S. Mulliken of U. Chicago, L.E. Orgel of Salk Institute, and M. Kasha of Florida State U. (e.g., JACS 85 2899) concerning charge transfer absorptions, W.C. Price of Kings College, London, and W. Kaye of Beckman Co., Fullerton, Calif. (e.g., Appl. Spectroscopy 15:89) concerning vacuum UV measurements, and J. Linnett of Queens College, Oxford, (e.g., JACS 83: 2643) concerning the electronic structure of molecules.

14. TECHNICAL PROGRESS IN FY 1965

Much of the activity during 1965 has been devoted to the problem of getting the work started. Preliminary results have shown that for adenine the protective power of some ions and molecules is directly proportional to the product of the intensity of the charge transfer absorption band in the ultraviolet region near 200 nm and the concentration. This observation will be tested further for this and other compounds.

A method for construction of 3-dimensional models of the electronic structure of molecules has been developed, based on the principle of tetrahedral spin-set correlation proposed by Dr. Linnett of Queens College, Oxford. The models appear to give insight into the electronic nature of ground and excited states of ions and simple molecules.

15. EXPECTED RESULTS IN FY 1966

It is anticipated that the study of *Neurospora* will be pursued and that it will be possible to determine which phases in its life cycle are particularly sensitive to radiation. Attempts will then be made to protect these phases from radiation damage by use of various materials which are considered to be possible protective agents.

The study of charge transfer bands in the near UV region of the spectrum will be continued, and attempts will be made to correlate the electronic configurations of the molecules with 3-dimensional models of their electronic structure. In this, the hypothesis that particular electronic structures are important in radioprotection will be tested. The work in the vacuum ultraviolet will be delayed until there is access to a vacuum spectrometer.

16. EXPECTED RESULTS IN FY 1967

It is hoped that it will be possible to purchase a vacuum spectrometer which will make possible the examination of charge transfer absorption bands in the vacuum ultraviolet, that is, in the wavelength region below about 195 nm. It is anticipated that the general program described for this project will be continued and that there will be emphasis on those aspects which are found to be most promising during the next year.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Medical Physics Instrumentation

3. AEC Budget Activity No.:
06-06-02

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Benedict Cassen

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3 1/4	3 1/4	3 1/4
(b) Other Tech.	0	0	0
Total	3 1/4	3 1/4	3 1/4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 34,600	$ 33,000	$ 34,100
(b) Materials & Services	4,000	4,000	4,000
(c) Indirect Expenses*	(3%) 30,700	(2%) 21,900	(2%) 22,400
Total	$ 69,300	$ 58,900	$ 60,500

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

None (A paper was read at the Berkeley meeting of the Society of Nuclear Medicine on the Depth of Focus of Converging Collimators.)

12. SCOPE OF THE PROJECT

The purpose of the Medical Physics Section activity is to develop new procedures and instruments that are especially applicable to currently important problems in radiobiological research and in nuclear medicine. Currently there are three major efforts in progress as well as other lesser developments.

The first, which is now essentially completed in its first phase, is that of exploring new possibilities of appreciably increasing gamma quantum utilization efficiency in radioisotope scanning. (See 14 below)

The second major effort consists in developing new procedures for the gross separation and characterization of viable cell suspensions into groups having different physical characteristics. Such methods, depending on differences of electrophoretic zeta potential, on size and shape characteristics, and density differences are being applied to separated lymphocytes and to age group separations of erythrocytes (see 14 below).

A third effort is being made to explore the possibilities of a mosaic screen of clear calcium tungstate rods to perform as a high absorption efficiency intensifying screen for use either with an image amplifier or directly with ultra high speed photographic film. Preliminary promising results have been obtained with a technetium 99m source. (See 14 below)

Some further experiments have been made to determine the advantages and feasibility of producing highly localized radiation lesions with an essentially penumbraless pencil-like x-ray beam being rotated over a large solid angle of entry with the center of rotation at the position of the desired lesion. (See 14 below)

13. RELATIONSHIP TO OTHER PROJECTS

1. High Quantum Utilization Scanner

This phase of development is in cooperation with the UCLA School of Medicine Neurosurgery Group and especially with Dr. Paul Crandall of that group. Some others working in the field of improving radioisotope imaging are:

Dr. Gordon Brownell, Massachusetts General Hospital, Boston. Drs. Bender and Blau, Roswell Memorial Institute, Buffalo, N.Y. Dr. H. Anger, Donner Lab. University of Calif., Berkeley, Calif. Dr. Beck, Argonne Cancer Hospital, Chicago, Ill., Dr. Harris, Oak Ridge National Laboratory.

2. Physical Cell Measurements and Separation

There are many investigators in this field; most closely at present is Howard Mel, Donner Laboratory, Berkeley, Calif.

3. Scintillation Hemodynamics

Dr. I. Mena, Catholic University of Chile, Santiago, Chile; Dr. W. Oldendorf, U.S. Veteran Hospital, Los Angeles, Calif.

14. TECHNICAL PROGRESS IN FY 1965

1. High Quantum Utilization Scanner

The high quantum utilization scanner was designed originally for optimum performance with iodine 131 and mercury 203. With the availability of technetium 99m, the clinical brain tumor scanning test program was switched to this isotope. Very fast scans can be made on patients. Patients with brain tumors have been scanned sectionally in order to take advantage of the shallow depth of focus of a large solid angle detector. The depth of the plane of maximum activity in the tumor area can be determined and approximate stereotaxic coordinates of the tumor can thereby be established. The application of the sectioning feature to other scanning problems besides brain tumors is being studied. A simple local television circuit has been set up to view out-of-focus dot scans with various contrast settings.

2. Physical Cell Measurements and Separations

A special linear amplifier was acquired and used between a Coulter aperture and a multichannel analyzer. This greatly improved the linearity, accuracy, reproducibility and statistical quality of cell size distribution results. (See Budget Activity 06-01-01)

Several developmental types of electrophoretic viable cell separators have been improved and tested. Recently, reproducible stabilized flow of a thin sheet of fluid was obtained by pumping the fluid through a 12 channel peristaltic pump. For electrophoretically separating viable cells the medium is of sufficiently high electrical conductivity that in order to obtain a high ffeld gradient it is necessary to dissipate a considerable amount of power as heat. An efficient cooling plate for the thin liquid film was developed by coating an aluminum water cooled plate with a thin film of fluorinated hydrocarbon (Kel-F).

A program has been initiated to continue work done for a Ph.D. thesis at the California Institute of Technology by Dr. R.C. Leif who is now working with us as a post-doctoral fellow in biophysics. Erythrocytes (and hopefully bone-marrow cell suspensions) are separated into groups of slightly different densities by a precision buoyant gradient technique.

15. EXPECTED RESULTS IN FY 1966

It is expected that a large series of patients will be run on the high quantum utilization sectioning scanner.

It is anticipated that a useful calcium tungstate mosaic intensifying screen can be developed to obtain direct images of technetium -99m distributions.

A program will be continued to still further improve and perfect the cell size spectra determination and electrophoretic cell separation procedure.

It is expected that techniques and instrumentation can be perfected to separate cell suspensions into groups of slightly different densities by a precision buoyant density gradient technique.

16. EXPECTED RESULTS IN FY 1967

It is anticipated that considerable improvements and simplifications can be made in radioisotope distribution imaging by the use of calcium tungstate mosaic screens.

It is expected that a program of developing methods of transferring stereotaxic scanning information to a 3-dimensional rotational radiation lesion producing system will continue.

It is expected that viable blood and bone marrow cells can be rapidly separated into groups by electrophoresis and buoyant density gradient methods, and that these methods can be applied to give diagnostic information on cancer and leukemia patients.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Chemical Toxicity of Rare Earths

3. AEC Budget Activity No.:
06-07-00

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Thomas J. Haley

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2 ½	2 ½	2 ½
(b) Other Tech.	0	0	0
Total	2 ½	2 ½	2 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 27,600	$ 27,500	$ 27,500
(b) Materials & Services	3,200	3,400	3,400
(c) Indirect Expenses*	(2%) 20,400	(2%) 21,900	(2%) 22,400
Total	$ 51,200	$ 52,800	$ 53,300

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Pharmacology and Toxicology of Praseodymium and Neodymium Chlorides, Haley, T.J., Komesu, N., Efros, M., Koste, L., and Upham, H.C., Toxicol. and Applied Pharmacol., 6, 614-620 (1964).

2. Pharmacology and Toxicology of Lutetium Chloride, Haley, T.J., Komesu, N., Efros, M., Koste, L. and Upham, H.C., J. of Pharm. Sciences, 53, 1186-1188 (1964).

3. Pharmacology and Toxicology of Europium Chloride, Haley, T.J., Komesu, N., Colvin, G., Koste, L. and Upham, H.C., J. of Pharm. Sciences, in press.

4. Pharmacology and Toxicology of Dysprosium, Holmium and Erbium Chlorides, Haley, T.J., Koste, L., Komesu, N., Efros, M. and Upham, H.C., Toxicol. and Applied Pharmacol., in press.

5. Clinical Toxicology, 4th Ed., C.H. Thienes and T.J. Haley, Lea & Febiger, Philadelphia, Pennsylvania, 1964.

12. SCOPE OF THE PROJECT

It is essential that more information be made available concerning chemical and physical agents which cause changes in the animal organism relative to its ability to maintain homeostasis. In this regard it is our objective to discover what changes can be brought about by both types of noxious agents in respect to acute and chronic effects. It is also necessary to understand the basic mechanisms involved in order to properly understand the problems which may arise through the application of therapeutic measures designed to counteract and/or correct the detrimental changes which may be caused by exposure to chemical or physical injury. Analysis of the changes occurring in normal physiological functions (blood pressure, respiration, growth, etc.) as well as in products produced in metabolism and excretion may give an insight into the basic defects caused by exposure to chemical or physical agents. The application of sensitive methods to the measurement of changes in known tissue constituents can give information to the sites of action of noxious agents.

13. RELATIONSHIP TO OTHER PROJECTS

Argonne National Laboratory; Iowa State College; Los Alamos Scientific Laboratory; MIG; New York University; NRDL; University of Rochester Atomic Energy Project; USAF Radiation Laboratory; UT-AEC; Various Sections of the University of California, Los Angeles, Department of Biophysics and Nuclear Medicine, Laboratory of Nuclear Medicine and Radiation Biology.

14. TECHNICAL PROGRESS IN FY 1965

With the completion of the pharmacology and toxicology of the rare earth elements, 59 through 71, a review will be published covering the total toxicological picture. Work will be initiated on germanium, rhenium, ruthenium and rhodium to establish the toxicological parameters necessary for the setting of individual industrial hygienic standards.

15. EXPECTED RESULTS IN FY 1966

Investigation will be made of local and systemic toxicological effects of other rare elements which are being utilized in the atomic energy program

either directly or indirectly. Such an evaluation of the chemical toxicity of the rare elements will be of assistance in establishing industrial hygienic guides for the protection of workers in the various industries employing the above elements.

16. EXPECTED RESULTS IN FY 1967

A program will be initiated as required for the study of new reactor coolants to be used with the heavy water reactors now being designed. Other rare elements of interest to the Atomic Energy Commission will be studied for their pharmacological and toxicological properties, particularly those aspects dealing with chronic exposure.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Biological Studies of Leukemia

3. AEC Budget Activity No.:
06-09-00

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Esther F. Hays, M.D.

8. Project Term:
From: 1955 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2 1/4	2 1/4	2 3/4
(b) Other Tech.	2	2	2
Total	4 1/4	4 1/4	4 3/4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 32,400	$ 33,000	$ 38,500
(b) Materials & Services	7,000	6,400	6,400
(c) Indirect Expenses*	(2%) 20,400	(2%) 21,900	(2%) 22,400
Total	$ 59,800	$ 61,300	$ 67,300

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Hays, E.F., Growth and Morphology of Mouse Lymphoma Cells in Diffusion Chambers, Proc. Soc. Exper. Biol. and Medicine, 117, 45-50, 1964.

2. Hays, E.F., Comparative Oncogenic Properties of Deoxyribonucleic Acid from Primary Tumors, and Polyoma Virus, Cancer Research, 24, 1741-47, 1964.

12. SCOPE OF THE PROJECT

The research in our laboratory at the present time is concerned with several facets of experimental leukemia in mice. First, we are studying the role of the thymic epithelial reticular cells in reconstituting lymphoid depleted, thymectomized mice. Ultimately, this project is designed to determine the role of these epithelial reticular cells in leukemogenesis. Neonatally thymectomized mice of a low incidence leukemia strain grafted with thymic epithelial reticular cells from a high incidence strain as well as those of a low incidence leukemia strain infected with the Gross leukemia virus and grafted with isogeneic thymic reticulum will be studied.

Secondly, a study is in progress to determine if tumor cell antigenicity can be demonstrated in spontaneous leukemias if mice of the AKR strain, and if this antigenicity is different from that associated with Gross virus induced leukemia in this same strain.

Thirdly, we are studying infectivity of viral nucleic acid from the Gross and Rauscher leukemia viruses as well as concluding several experiments designed to demonstrate leukemic transformation of normal lymphoid cells with deoxyribose nucleic acid extracted from leukemic cells of mice with the spontaneously developing disease.

13. RELATIONSHIP TO OTHER PROJECTS

Studies of experimental leukemia in mice are being undertaken by many investigators. The following list mentions a few whose work has been of invaluable assistance to this project. Dr. L. Gross, V.A. Hospital, Bronx, New York; Dr. H. Kaplan, Stanford University Medical School; Drs. Maloney, Law, Stewart and Rauscher at the National Cancer Institute, Bethesda, Md.; Dr. J. Furth, Francis Delafield Hospital, New York, New York; Dr. J.F.A.P. Miller, Chester Beatty Institute, Pollards Wood, England and Dr. Britta Wahren, Institute for Cell Research, Karolinska Institute, Stockholm, Sweden.

Here at UCLA Drs. Paul Terasake of the Department of Surgery and Dr. W.H. Hildemann of the Dept. of Medical Microbiology and Immunology have given invaluable assistance and advice in the field of cellular immunology.

14. TECHNICAL PROGRESS IN FY 1965

Several studies have been completed in which an attempt was made to induce a transformation of normal to leukemic cells utilizing deoxyribose nucleic acid prepared from leukemic tissues of mice with spontaneous disease.

These results can be summarized by saying that utilizing both in vitro (incubating cells with DNA and then innoculating them) and in vivo (innoculating DNA into thymic grafts placed in suckling mice thymectomized at birth) methods it has not been possible to effect a transformation. Also when both DNA and RNA were extracted from tissues of mice with virus induced leukemias known to contain virus and innoculated into suckling mice, no leukemias resulted.

A histological study of lymphoid tissues of neonatally thymectomized mice has been completed and has shown that lymphoid development is arrested at one week of age and this lymphoid atrophy, particularly in the intermediate zone of lymph nodes, but also in Peyers patches and splenic follicles, continues to be present and becomes more marked with the passage of time. Also we have demonstrated an increase and persistence of extramedullary hematopoesis in lymph nodes of mice thymectomized when newborn. We have found slightly less lymphoid atrophy in neonatally thymectomized mice of a strain with a high incidence of lymphocytic leukemia strain treated in the same manner.

15. EXPECTED RESULTS IN FY 1966

AKR (high incidence leukemia) strain mice given repeated innoculations of leukemia cells from isogenic mice with both virus induced and spontaneous lymphocytic leukemia - will have measurements of cytotoxic antibodies against the tumor cells performed on their serum. These measurements will be correlated with the growth properties of small doses of these cells in AKR mice.

The ultimate aim of the study is to see if the leukemic cells of virus induced tumors have different antigenic properties than those from animals with the spontaneous disease.

Radiation induced and virus induced leukemia in C3HeB mice will be studied in a similar manner.

Studies of thymic epithelial cell grafts from mice of a high incidence leukemia strain will be carried out using neonatally thymectomized C3HeB mice as donors. The grafts are prepared by placing a neonatal thymus in a diffusion chamber for one week. The thymuses treated in this manner lose their cortical cells but the epithelial reticular cells persist. The ability of these grafts to restore lymphoid tissue and immunologic capacity will be studied as well as their leukemogenic properties in these animals.

16. EXPECTED RESULTS IN FY 1967

During this period we will utilize cytogenetic techniques to study leukemic cells from mice with a marker chromosome (T_6). We will innoculate small numbers of marked leukemia cells into susceptible animals, i.e. fibroid leukemias into the parental strain without the markers, and study their growth properties in thymectomized and intact adult mice. This study will be initiated to learn more about the role of the thymus in leukemogenesis; i.e. does the thymus sequester leukemic cells antigenically different from the host, and what is fate of leukemic cells innoculated into thymectomized animals? It is a well known fact that thymectomy prevents lymphoid leukemia induction by virus and irradiation and in these two instances the leukemic transformation is induced in the cells of the host and the thymus is necessary for the actual development of the disease. We hope to elucidate its role regulating the spread of leukemic cells from an external source utilizing the studies outlined above.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Clinical Nuclear Medicine

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA and Harbor General Hospital

7. Person in Charge:
George V. Taplin, M.D.

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	5 1/2	5 3/4	5 1/2
(b) Other Tech.	1 1/2	1 1/2	1 1/2
Total	7	7 1/4	7

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 67,100	$ 70,400	$ 76,400
(b) Materials & Services	14,700	12,400	13,400
(c) Indirect Expenses*	(5%) 51,200	(5%) 54,800	(5%) 56,100
Total	$133,000	$ 137,600	$ 145,900

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

Taplin, G.V., Johnson, D.E., Dore, E.K., Kaplan, H.S.: Suspensions of radioalbumin aggregates for photoscanning the liver, spleen, lung and other organs, J. Nucl. Med. 5:259-275 (1964).

Taplin, G.V., Johnson, D.E., Dore, E.K. Kaplan, H.S.: Lung photoscans with macroaggregates of human serum radioalbumin (Experimental basis and clinical trials), Health Physics 10:1219-1227 (1964).

Dore, E.K., Taplin, G.V., Johnson, D.E., Cockett, A.: Experimental basis of quantitative radiorenography for evaluating renal ischemia, J. Nucl. Med. (in press).

Taplin, G.V., Dore, E.K., Johnson, D.E.: Hepatic blood flow and reticuloendothelial system studies with radiocolloids. Dynamic Clinical Studies with Radioisotopes, TID-7678, Proc. ORINS Symp. in Med. #8, 285-317(1964).

Taplin, G.V., Dore, E.K., Garcia, E.J., Johnson, D.E.: Reticuloendothelial system studies with radio colloids, Proc. IX Cong. Internat. Soc. Hemat., Universidad Nacional Autonoma de Mexico, Mexico City, Mexico, 3:439-449(1964).

Taplin, G.V., Johnson, D.E., Kennady, J.C., Griswold, M.L.: Lung and brain scanning with macroradioalbumin aggregates, Scientific Exhibit, Clin. Cong. Amer. Coll. Surg., Chicago, Illinois, Oct. 5-9, 1964.

Taplin, G.V., Johnson, D.E., Dore, E.K., Poe, N.D., Greenberg, A. Pulmonary embolism and bronchogenic carcinoma. Detection by radioisotope scanning (scientific exhibit, J. Nucl. Med. 5(1965) (abstract).

Dore, E.K., Johnson, D.E., Taplin, G.V.: Rapid brain and lung scanning. With modified conventional equipment, J. Nucl. Med. 5(1965) (abstract).

Swanson, L.A., Dore, E.K., Johnson, D.E., Taplin, G.V.: Correct interpretation of renograms and scans in unilateral kidney displacement, J. Nucl. Med. 5 (1965) (abstract).

Poe, N.D., Taplin, G.V.: Physiological changes in pulmonary blood flow evaluated by lung scanning, J. Nucl. Med. 5 (1965) (abstract).

Taplin, G.V., Poe, N.D., Greenberg, A.: Lung scanning following radioisotope inhalation (Techniques and potential applications), J. Nucl. Med. 5 (1965) (abstract).

12. SCOPE OF THE PROJECT

In modern medicine diagnostic radioisotope techniques are playing an increasingly important role. Early diagnosis is stressed as the first essential to successful medical and surgical treatment. The major goal of this project is to extend the application of radioisotopes in medical diagnosis. New tracer procedures must meet certain requirements. They should reveal reliable information which is otherwise impossible, impractical or hazardous to obtain, or provide supplementary data which aid evaluation of other diagnostic procedures. Scintillation counting techniques are unique in that they may be performed externally to the body and permit measurement of tracer as it enters and leaves internal organs. Also the size, shape and position of various organs may be determined by photoscanning and abnormalities such as tumors, cysts or abscesses may be detected as areas of

either increased or decreased tracer concentration. In addition tracer methods allow assessment of organ blood supply with relative simplicity. Such information alone has diagnostic value and also assists interpretation of structural abnormalities seen radiographically and of functional disturbances found biochemically.

Presently the clinical work in nuclear medicine includes tracer studies of organ function, structure and hemodynamics in conjunction with standard diagnostic procedures. Patients with diseases of the RES, kidneys, liver, heart, lungs and brain are being investigated. Parallel studies are made in animals. This dual approach has led to the development of several valuable radioisotope procedures in the past and to their continuing improvement. New applications developed during the past year include preparation of radioalbumin aggregates of selected particle size ranges. Aggregates less and 1μ are useful as test agents for visualizing the heart, spleen and stomach by photoscanning. Aggregates in the 5-25μ range are removed during the first passage through the lungs and permit visualization of this organ. Particles somewhat larger, 20-50μ appear to be suitable for scanning one hemisphere of the brain or the kidneys following intra arterial injection in conjunction with routine angiography.

13. RELATIONSHIP TO OTHER PROJECTS

Studies are being made at nearly all of the major universities and medical centers in the USA; at the Institute of Nuclear Studies, Oak Ridge; Brookhaven National Laboratory; in various foreign medical centers such as the University of Lund, Malmo, Sweden; Curie Institute, Paris; 2nd Medical Clinic University of Vienna; University of Heidelberg, Germany; Univeristy of Athens, Greece; University of Pisa, Italy; Institute of Cancer Therapy, Lisbon, Portugal; Institute of Cardiology, Mexico City, Mexico; Catholic University, Santiago, Chile; Institute for Nuclear Studies, Sao Paolo, Brazil; University of Geneva, Switzerland; National Institute of Radiological Sciences, Chiba, Japan; Imperial University of Tokyo, Japan; Atomic Energy Agency and Clinical Hospital, University of Buenos Aires, Argentina; Guys Hospital, London, and the French Atomic Energy Agency establishment at Orsay.

14. TECHNICAL PROGRESS IN FY 1965

Lung scanning Procedures: The technique of lung visualization by scanning after the intravenous injection of radioalbumin macroaggregates has been improved and applied to numberous clinical problems with complete safety in over 300 patients. The scan image of the lung represents the plumonary arterial blood flow pattern and thereby provides a relatively simple means for demonstrating regional alterations of blood flow distribution in a variety of lung diseases and also the effects of posture and drugs. Serial lung scans and roentgenorgraphic studies in pulmonary embolism demonstrate the unique diagnostic value of the lung scan. It can detect the site and magnitude of arterial obstruction, long before roentgenological signs are recognizable and allows one to follow the natural course of this disease and the results of medical or surgical therapy. The I.V. lung scan also has usefulness in evaluating pulmonary function in emphysema, tuberculosis and bronchiectasis, because ischemic lung tissue is not capable of efficient gas exchange.

Two new techniques of lung scanning are being developed on the premise that the pulmonary distribution of inhaled radioisotopes should indicate the site and degree of partial obstruction in the bronchial tree. One procedure involves the inhalation of radioaerosols of albumin I^{131}, rose

bengal I^{131}, colloidal gold ^{198} and chlormerodrin Hg^{197}, all of which are retained in the respiratory tract long enough for scanning purposes. A positive pressure respirator-nebulizer apparatus with suitable exhaust manifold, filter and exhaled air vent permit aerosol inhalation without contamination of the test area. Partial bronchial obstruction is detectable in the scan by an area of increased radioactivity at the site of the obstruction. Normally the I.V. and aerosol inhalation lung scan images are nearly identical but the latter represents the distribution of retained particles and not the true ventilatory pattern.

A second type of inhalation scanning procedure is being developed to determine pulmonary ventilation and for the detection of bronchial obstruction. This procedure is performed during the re-breathing of Xe^{133}-oxygen mixtures using a modified basal metabolism machine. With nuclides of two widely separated energies, the I.V. and inhalation scans can provide data on pulmonary arterial blood flow and ventilation at almost the same time.

New Developments in Radiorenography and Renal Scanning: The I^{131} ortho iodo hippurate (OIH) renogram plus the 15 minute excretion of OIH provides quantitative data on differential and total renal blood flow, urine flow and/or interference with urine drainage. Renal scanning with Hg^{197} chlormerodrin adds information on kidney size, shape, position and amount of functional kidney tissue. However, these procedures do not measure another important function of the kidney, namely glomerular filtration. This parameter is estimated by the clearance of inulin but the technique and chemical assay are cumbersome, and complicated. Two urographic contrast agents, hypaque and conray (iothalamate) labeled with radioiodine are excellent inulin substitutes. Their use in renography together with OIH is being explored to develop a dual tracer renogram technique for measuring differential blood flow and glomerular filtration simultaneously.

Kidney scanning with radioalbumin macroaggregates has been shown to be feasible and safe provided the dose of carrier albumin is kept below 1 mg/kg and particle size is maintained in the range of 10-60μ. Scans of kidney sections following renal artery injection of these particles demonstrate that the renal arterial blood flow is greatest in the cortical region of the kidney.

15. EXPECTED RESULTS IN FY 1966

Emphasis will be placed on further development of the intravenous and inhalation lung scanning techniques for evaluating pulmonary arterial blood flow and the patency of the bronchial tree. The I.V. and inhalation scan data will be correlated with roentgenographic studies and differential spirometry for estimating total and regional pulmonary function in chronic obstructive bronchopulmonary disease (emphysema, bronchiectasis, tuberculosis). Clinical studies will include further investigations of the natural course of pulmonary embolism, acute pneumonia, post operative pneumonia and atelectasis. Long term studies of pulmonary tuberculosis are being continued to relate changes in pulmonary blood flow to the natural course of the disease and to the effectiveness of specific therapy.

Kidney studies will include further development and clinical application of the double tracer technique for simultaneously estimating differential renal blood flow (hippuran I^{125}) and glomerular filtration (iothalamate I^{131}) in hypertensive patients with chronic phelonephritis and renal artery occlusive disease. The double tracer procedure will also be tried in acute glomerular nephritis and in the nephrotic syndrome. In the renal hyper-

tension program, correlative data on renal blood flow and pressure gradient measurements determined at operation will be correlated with the renogram and renal scan findings.

Liver and RES studies will be continued using colloidal radioalbumin aggregates for liver-spleen scanning and for estimation of liver blood flow and phagocytic function. In addition colloidal Tc^{99m} will be prepared and investigated for the same purpose because of its low energy, short half life and small radiation exposure.

16. EXPECTED RESULTS IN FY 1967

Organ Scanning: The organ scanning program using radioalbumin macroaggregates and Tc^{99m} preparations will be expanded with the acquisition of a fixed position camera-type scanner. Such scanners permit dynamic visualization of the turnover of organ specific tracers minute by minute or faster with small organs. The serial scan images can display functional abnormalities of whole organs and areas of localized disease. The capacity to visualize an organ rapidly permits the use of nuclides in particulate form which are only transiently retained in capillary beds. For example, in brain scanning with albumin macroaggregates (10-60 μ) smaller particle size suspensions (10-30 μ) are more quickly removed, ($T_{1/2}$ = 20-30 minutes) and are far less likely to cause central nervous system injury than the larger preparations presently required. The ability to obtain organ blood flow patterns by rapid scanning following transient arteriolar blockade, could extend the application of this technique to many of the organs and body regions now being studied by arterial angiography and provide additional diagnostic information with less hazard from reactions or radiation exposure.

Dynamic Tracer Studies: The assessment of liver and kidney blood flow and function of these organs by simultaneous external monitoring will be continued using organ specific agents labeled with nuclides of widely separated energy spectra. The dynamic tracer studies and scan results will be correlated with standard diagnostic procedures and with direct measurements of blood flow and blood pressure gradients at operation.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Basic Nuclear Medicine

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
George V. Taplin, M.D.

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4	4	4
(b) Other Tech.	1	½	½
Total	5	4 ½	4 ½

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 30,300	$ 30,800	$ 36,300
(b) Materials & Services	9,500	8,800	8,800
(c) Indirect Expenses* (2%)	20,400	(3%) 33,000	(2%) 22,400
Total	$ 60,200	$ 72,600	$ 67,500

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS IN FY 1965

Dore, E.K., Taplin, G.V., Johnson, D.E., Cockett, A.: Experimental basis of quantitative radiorenography for evaluating renal ischemia, J. Nucl. Med. (in press).

Taplin, G.V., Dore, E.K., Garcia, E.J., Johnson, D.E.: Reticuloendothelial system studies with radio colloids, Proc. IX Cong. Internat. Soc. Hematil., Universidad Nacional Autonoma de Mexico, Mexico City, 3:439-449 (1964).

Poe, N.D., Taplin, G.V.: Physiological changes in pulmonary blood flow evaluated by lung scanning, J. Nucl. Med. 5 (1965) (abstract).

Taplin, G.V., Poe, N.D., Greenberg,A. :Lung scanning following radioisotope inhalation (Techniques and Potential Applications), J. Nucl. Med. 5 (1965) (abstract).

12. SCOPE OF THE PROJECT

The activities of this project are related directly to those of the clinical nuclear medicine section. New tracer procedures are studied first in animals under controlled conditions to establish their validity as indicators of disturbed physiological functions. Pathological states are induced experimentally and the associated physiological disturbances are assessed by standard methods along with the tracer procedures. The animal work permits investigation of many parameters which are not possible to study directly in man. For example, results of indirect measurements (electromagnetic flowmeters and/or actual collection of blood flowing from an organ). Animal studies also provide means for investigating the effects of drugs, hormones, toxic or sensitizing agents and surgical manipulation on specific organ functions. Simultaneous direct measurements permit quantitative evaluation of indirect tracer techniques.

Another function of this section is to develop new and/or improved tracer materials. For example, colloidal suspensions of human serum albumin I^{131} are prepared in this section. Particle size measurements, biological tests, radioassay for free and bound I^{131} and sterility tests are performed, before such preparations are used in the clinical section. Studies of the relative antigenicity of this material in man and in various species are conducted using agar diffusion techniques. Thus this section provides the proving ground for new ideas, techniques and test materials and the means for further development and quantitative evaluation of clinically applicable tracer methods.

13. RELATIONSHIP TO OTHER PROJECTS

Similar studies are being made at the University of Lund, Malmo, Sweden; at the Karolinska Institute in Stockholm, Sweden; at the University of Heidelberg, Germany; Brookhaven National Laboratory; Veterans Administration Hospital, Los Angeles.

14. TECHNICAL PROGRESS IN FY 1965

Renal Studies: Three types of basic studies related to kidney function were conducted this year in support of the clinical program on renal hypertension. The effects of mercury bichloride poisoning on renal blood flow in rabbits was studied by the standard clearance methods, radiorenography, direct blood flow measurements and the kidneys were examined histologically to correlate cellular damage with the physiological findings.

A parallel relatioship was shown between the degree of cellular damage and impairment of renal blood flow as measured directly and by the clearance techniques. Extraction efficiency for hippuran in tracer quantities was approximately 83% in normal animals but was reduced in proportion to the severity of cellular damage as determined histologically. The renogram as an indicator of individual renal blood flow reflected not only the tubular damage in the poisoned animals but also the impairment of blood flow and extraction efficiency. These data further substantiate our current concept of the renogram as an indicator of individual renal blood flow.

The hippuran renogram is not an indicator of glomerular function. Therefore studies were made with I^{131} labeled iothalamate, a urographic contrast agent which is supposedly a good inulin substitute. Clearance studies and extraction efficiency measurements were made in dogs. This material is extracted by the kidneys with an efficiency of approximately 25% which is nearly identical to that of inulin. Prior administration of large doses of PAH fail to alter the extraction efficiency as measured by simultaneous assay of renal vein and artery blood samples. Furthermore, PAH injections did not alter the contour of the iothalamate I^{131} renogram, when given immediately prior to the tracer.

Renal Arteriolar Blockade with Radioalbumin Macroaggregates: Intra arterial injection of 10-60 μ size albumin aggregates via a femoral artery catheter produced renal blood flow reduction and renograms having the same contour as those found following renal artery constriction when a similar degree of blood flow reduction was produced. In both instances, the slope of the renogram's second segment is depressed and the transit time is increased.

In addition, the macroaggregates were used to demonstrate the intrarenal distribution of blood flow. When such particles are injected directly into the dog's renal artery and the kidney is removed within 30 seconds, the sites of arteriolar entrapment can be determined by radioisotope scanning. The scan image of a transverse section of the kidney represents the pattern of arterial blood flow and shows that the main flow is to the cortical region. These findings are in substantial agreement with those of Dr. Clifford Barger of Harvard University who obtained the same information by external monitoring techniques using Xe^{133} by renal artery injection.

Pulmonary Function Studies: Two new techniques for visualizing the lung fields following the inhalation of radioisotopes are being developed. In the first method various gamma emitting tracer materials are inhaled in aerosol form using positive pressure respirator-nebulizer equipment. The scan image of the lungs so obtained represents the distribution of inhaled particles and their deposition within the bronchial tree. With partial bronchial obstruction, an area of increased radioactivity is found at the point of obstruction and the area supplied by this bronchus shows relatively reduced activity. Suitable test agents for this method include albumin I^{131}, rose bengal I^{131}, Hg^{197} labeled chlormerodrin and colloidal gold ^{198}. The aerosol inhalation scan images do not represent true pulmonary ventilation. Therefore, a second method is being devised, wherein the lungs are scanned during continuous re-breathing of Xe^{133}-oxygen mixtures. For this procedure a modified basal metabolism machine is employed. Areas of reduced activity in the gas inhalation scan represent impaired ventilation capacity. The intravenous and inhalation scans may be performed during the same visit if a low energy tracer is used for inhalation and a high energy one for the intravenous technique. This dual isotope procedure permits evaluation of arterial blood flow and ventilatory capacity almost simultaneously.

RADIOALBUMIN MACROAGGREGATES: Recent improvements were made in the method of preparation and the control of particle size distribution. Over 100 batches of macroaggregates were examined microscopically for particle size distribution using a filar micrometer. A bioassay method was developed to determine the relative deposition in the lungs vs the liver, five minutes following intravenous injection. When the bioassay showed ratios of lung to liver radioactivity greater than 10 the quality of human lungs scans was uniformly satisfactory.

EXPECTED RESULTS FY 1966

Renal Studies: The program of autoradiography begun last year will be continued. The intrarenal localization and distribution of hippuran I^{125} iothalamate I^{125} and chlormerodrin Hg 197 will be determined. Autoradiographs will be made of kidney sections removed at specific intervals following tracer injection to determine the metabolic pathways of these agents at the cellular level. These studies will be made in conjunction with radiorenograms performed with these three test materials to further clarify the meaning of the renogram in relation to the type of test agent used and its metabolic pathway.

With the acquisition of a four channel dynamic analyzer dual tracer renograms will be performed simultaneously using nuclides of widely different energies such as I^{131} and I^{125} labeled test materials, one of which is totally filtered, the other mainly secreted by the kidney. The double tracer renogram technique will be studied in experimental conditions wherein either filtration capacity or tubular transport function is altered.

Tracer Studies of Pulmonary Function: Lung scanning studies following intravenous administration of macroaggregates and/or inhalation of radioaerosols will be continued with special emphasis on investigating the action of various drugs on the pulmonary circulation and on the release of bronchiolar spasm. The preliminary work on inhalation scanning during the re-breathing of radio Xe^{133}-oxygen mixtures will be extended to determine more fully the normal pulmonary ventilatory patterns as well as the effects of partial and total bronchial obstruction on the ventilatory pattern. Various disease states will be simulated in the dog and studied by the dual (inhalation-intravenous) scanning technique to help evaluate clinical results in patients with chronic obstructive bronchopulmonary disease.

Preparation of New Tracer Test Materials: Attempts will be made to devise methods for preparing colloidal albumin labeled with Tc^{99m} for liver-spleen scanning. The colloidal Tc^{99m} labeled aggregates will be converted to macroaggregates by pH adjustment and further heating for use in lung and brain scanning and for other studies of transient arteriolar blockade. In addition, further attempts will be made to improve the preparation of aggregated albumin in respect to better control of particle size distribution and to devise electronic methods for measuring the number of particles per milliliter using a Coulter counter and a 512 channel analyzer now available in the radiobiology division. All available colloidal preparations will be studied again using the new electron microscope operated by the biophysics division.

16. EXPECTED RESULTS IN FY 1967

The previous programs on basic investigations in renal physiology, pulmonary function and preparation of new tracer test materials all will be continued. It is likely that emphasis will be placed on the general technique

of organ scanning following temporary arteriolar blockade using gamma labeled protein aggregates. With the acquisition of a camera-type rapid scanner particle size of the test materials may be adjusted downward to shorten the time of their retention in the arteriolar capillary beds being studied. With smaller size preparations visualization of the myocardium becomes feasible and a program on myocardial scanning will be instituted. Another application to be tried first in animal studies is the evaluation of the integrity of the circulation to the extremities. There is a need for improved diagnostic techniques for both the cardiac and peripheral vascular disease problems. With the availability of rapid scanning techniques, attempts will be made to develop new tracer test materials with short half-life, low energy and rapid organ turnover as diagnostic tools for determining disturbances of organ function on a dynamic basis.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Hemodynamics

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
Harbor General Hospital and UCLA

7. Person in Charge:
George V. Taplin, M. D.

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	2	2	2
(b) Other Technical	1	1	1
Total	3	3	3

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 24,300	$ 26,400	$ 28,400
(b) Materials & Services	8,500	10,200	10,200
(c) Indirect Expenses*	(2%) 20,400	(2%) 21,900	(2%) 22,400
Total	$ 53,200	$ 58,500	$ 61,000

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

Taplin, G.V., Johnson, D.E., Dore, E.K., Kaplan, H.S.: Lung photoscans with macroaggregates of human serum radioalbumin (Experimental basis and clinical trials),Health Physics 10:1219-1227 (1964).

Johnson, D.E., Lui, C.K., Akcay, M.M., Taplin, G.V.: Radiopulmonary cardiography for measurement of central mean transit time and its arterial and venous subdivisions, Dynamic Clinical Studies with Radioisotopes, TID-7678, Proc. ORINS symp. #8 in Med. 249-266 (1964).

Taplin, G.V., Johnson, D.E., Kennady, J.C., Griswold, M.L.: Lung and brain scanning with macroradioalbumin aggregates, Scientific Exhibit, Clin. Cong. Amer. Coll. Surg., Chicago, Ill., Oct 5-9, (1964)

Akcay, M.M., Johnson, D.E., Taplin, G.V.: Radiotracer dilution studies in single tube systems. Significance to radiocardiography, J. Nucl. Med. 5, (1965). (abstract).

Kennady, J.C., Taplin, G.V.: Further brain scanning studies with radioalbumin aggregates, J. Nucl. Med., 5 (1965) (abstract).

Taplin, G.V., Kennady, J.C., Noblin, R.D., Griswold, M.L., Yamaguchi, M.: Arteriolar blockade studies with radioalbumin macroaggregates, exhibit J. Nucl. Med. 5 (1965) (abstract.)

12. SCOPE OF THE PROJECT

This section was established to broaden the scope of the sections on basic clinical nuclear medicine. Its objectives are to develop improved tracer techniques for the detection and evaluation of abnormal cardiocerebral and cardiopulmonary hemodynamics. Externally performed scintillation counting methods have the distinct advantage of simplicity, safety and of testing under physiological conditions. Cerebral and pulmonary angiography and other radiographic and neurosurgical procedures used to evaluate vascular lesions, are far more complicated and involve difinite hazards. Morphological information of great diagnostic value is obtained radiographically and by photoscanning. However the physiological significance of structural defects is frequently difficult to ascertain. The tracer procedures give valuable supplementary information in respect to underlying physiological disturbances.

The research activities of this section include controlled investigations in animals wherein clinical disorders are simulated. Related clinical studies are made and tracer techniques are modified on the basis of the animal data plus results in patients. The goals of the section are to develop reliable external monitoring procedures for evaluation of abnormalities of the cardiocerebral and cardiopulmonary circulation. A non-traumatic method for determining relative flow to the two hemispheres of the brain is especially needed today because recent progress in neurovascular surgery has brought dramatic cures to many totally disabled patients who were previously not considered for surgical treatment. Likewise in the area of cardiopulmonary disease, especially in the problem of pulmonary embolization and infarction, detection of regional disturbances in pulmonary blood flow and their localization by photoscanning could enhance the diagnostic ability of the physician because current radiographic procedures frequently fail to detect small lesions, especially during the first 24 hours. Early detection

of small pulmonary emboli could save many lives by directing the attention of the physician to this common and serious pulmonary complication, to institute appropriate medical and/or surgical treatment.

13. RELATIONSHIP TO OTHER PROJECTS:

Similar studies are being conducted at the Veterans Administration Center in Los Angeles by Drs. Oldendorf and Cassen and at the UCLA Center for Health Sciences by Drs. Crandall and Cassen; in Sweden by Drs. Hedlung Ljunggren and Regenstrom; similar studies on pulmonary scanning are under way at Johns Hopkins Hospital by Dr. Henry Wagner; at the University of Michigan by Dr. Beierwaltes, and at the Bowman-Gray Medical School, Winston-Salem, No. Carolina by Dr. Quinn.

14. TECHNICAL PROGRESS IN FY 1965

Experimental Brain Hemisphere Scanning in Primates: Long term cerebrovascular toxicity studies are being continued in primates because brain hemisphere scanning with radioalbumin aggregates has great potential diagnostic value and preliminary investigations last year suggested a 70-100 fold margin of safety. Sixteen monkeys have received multiple intra carotid injections of 10-100 μ size aggregates. Only two animals who were given more than 2 mg of carrier albumin had evidence of transient CNS injury, the others had no detectable impairment by EEG or by subsequent scanning with Hg^{197} chlormerodrin. They also had no behavioral changes or evidence of motor weakness. Five animals sacrificed after multiple injections showed no microscopic differences in brain sections between the control and injected hemispheres. On the basis of relative brain weights between monkey and man of 1 to 14 and the safety of multiple injections of 2 mg doses or less in the monkey, normal man should tolerate approximately 28 mg of the 10-100 μ size aggregates. The safety factor in man should be at least 100 fold because hemisphere scans can be performed with 0.28 mg of radioalbumin aggregates or less. Studies in the last few months using high specific activity (1000 μ c/mg albumin I^{131}) with particles no larger than 60 μ showed satisfactory scans in the monkey. With this smaller size distribution of the test material, the potential hazard should be further reduced. Clinical trials will be initiated in selected patients provided the histological examinations of the remaining animals also show no evidence of cerebral vascular abnormality in the injected hemispheres.

Radiotracer dilution studies: A systematic series of experiments was conducted with single tube model systems to more clearly define the variables affecting the externally recorded dilution curve such as that obtained by external monitoring over the heard in radiocardiography. The parameters of an indicator dilution curve are affected by three main variables: flow, dilution volume and detected volume. The area under the curve is not only inversely proportional to flow but is found to be also directly proportional to detected volume. Mean transit time and peak to peak time are not affected by the detected volume but configuration time is affected by both dilution and detected volumes. Mean transit times are considerably longer when calculated from dilution curves registered at 500 msec time intervals than those registered at 50 msec. As much as an 18% error is introduced if one uses instrumentation incapable of registering rapidly changing events with high precision. From the model studies it was shown that peak to peak time may replace mean transit time for volume measurements by applying a small correction factor. These results from model systems studies are being applied to the calculation of cardiac output and right heart blood volume from undistorted radiocardiograms obtained following the administration of 10-100 μ size radioalbumin macroaggregates.

Arteriolar Blockade studies: The mechanisms of arteriolar-capillary entrapment and release of 10-60 μ size albumin particles were observed directly in the exteriorized rabbit's omentum preparation. Direct observations at magnifications of 100 and 150 fold were made following injection of the stained aggregates following injection via a femoral artery catheter. Large aggregates are first trapped in the small arterioles. Particles are moved progressively forward by cellular bombardment. The vessel slowly dilates, blood cells and plasma move around the aggregate reducing its size by displacement of small fragments. The aggregate is then carried futher down the vessel and this process is repeated. In other vessels flow in the arteriole suddenly reverses its direction and the aggregate is carried into other vessels, possibly as a result of changing intra arterial pressure. Thus it appears that the passage of these malleable aggregates through the arterioles is accomplished by cellular bombardment and fragmentation and by continuous forward and backward movements within the arterioles until the aggregates are converted into smaller sizes and elongated forms capable of traversing the capillary lumina. Colored movies were taken to record these phenomena and will be presented in an exhibit at the National Society of Nuclear Medicine's 13th. annual meeting in Bal Harbour, Florida next June. Similar observations are being made in the dog brain preparation following common carotid artery injection of the same material.

15. EXPECTED RESULTS IN FY 1966

Brain Hemisphere Scanning: Further studies are planned in primates to gain further information on the relationship between particle size and the duration of arteriolar retention, not only in the capillary beds of the cerebral cortex but also in those of the extremities, kidneys and lungs. Clinical application of macroaggregate brain hemisphere scanning will be initiated first in selected patients having either inoperable brain tumors or advanced cerebrovascular disease and short life expectancy. In the first clinical trails high specific activity albumin aggregates with particle size no larger than 60 μ will be used cautiously. Further clinical application will be extended only if the first clinical trials are completed without immediate reactions or subsequent evidence of central nervous system injury. Furthermore, the procedure will be used as a screening test independently and not in conjunction with cerebral angiography. Further study in monkeys is needed to demonstrate the safety of injecting the particulate material simultaneously or shortly after the injection of angiographic test material.

Cardiocerebral Hemodynamics: With the acquisition of the four channel dynamic analyzer the cardiocerebral hemodynamic studies will be resumed to determine the effects of surgically produced cerebrovascular lesions and the effect of progressive internal carotid artery occlusion on ipsilateral cerebral blood flow. In these studies I^{125} albumin and/or hippuran and Tc^{99m} labeled albumin will be employed. Dilution curves over the heart and each cerebral hemisphere will be registered following the rapid intravenous injection of the tracer. Tracer results on differential hemisphere blood flow will be correlated with direct measurements made with electromagnetic flowmeters. Such experiments are needed to establish the validity of the cerebrogram procedure before resuming further clinical investigations.

Further Development in Quantitative Radiocardiography: Radiocardiograms from different patients cannot be compared quantitatively even in normal subjects because of differences in body size and in the amount of interposed tissue between the heart and the detector. From phantom studies the amount of interposed tissue can be calculated if one injects a test material of two nuclides of widely different energy spectra such as I^{125} and I^{131}. The ratio

of the count rates of the two nuclides at selected points in the curve permit calculation of the thickness of interposed tissue. The greater the amount of absorber between the heart and the detector the higher the I^{131} to I^{125} count rate ratio. By using this principle and doubly labeled radioalbumin macroaggregates attempts will be made to obtain the absorption factor and thereby eliminate one of the main uncontrolled variables in quantitative radiocardiography. Studies of this type will be made first in dogs and then extended to patients with a variety of cardiopulmonary abnormalities.

16. EXPECTED RESULTS IN FY 1967

It is anticipated that the clinical brain scanning program will be well underway. Brain hemisphere scan results will be correlated with the clinical and neurological findings with cerebral angiography, electroencephalography and with the findings at operation and/or autopsy. It is hoped that by this time, camera type rapid scanning equipment will be available and with the use of smaller size particulate test material and evidence of safety from prior experience, the program could be extended to the evaluation of a wide variety of cerebrovascular disorders. Likewise, with further development of the dynamic external monitoring techniques for cardiopulmonary and cardiocerebral applications, much of the effort will be placed on evaluation of these techniques in clinical practice.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:
Nuclide Metabolism

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Norman S. MacDonald

8. Project Term:
From: 1955 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	4 ½	5	5
(b) Other Tech.	1	1	1
Total	5 ½	6	6

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 55,900	$ 58,300	$ 60,300
(b) Materials & Services	9,400	10,800	10,800
(c) Indirect Expenses*	(4%) 41,000	(4%) 43,800	(4%) 44,700
Total	$106,300	$112,900	$115,800

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

MacDonald, N.S., Ibsen, K.H. and Urist, M.R.: Effect of tetracycline on retention of calcium and strontium in rodents, Proc. Soc. Exptl. Biol. and Med. 115:1125 (1964).

MacDonald, N.S., Figueroa, W.G. and Urist, M.R.: Short term retention of strontium-85 and estimation of initial strontium-90 body burdens in humans, USAEC Report UCLA-12-538, TID-4500 (1964) and Health Physics 1965 (in press).

MacDonald, N.S., Hamel, R., Hepler, M., and James, E.: Comparison of ^{95}Zr and ^{95}Nb Distributions in maternal and fetal rabbit tissues, Proc. Soc. Exptl. Biol. Med., 1965 (in press).

12. SCOPE OF THE PROJECT

The accumulation of radioactive isotopes within the body can present a serious hazard to human health. On the other hand, many such nuclides can serve as very valuable tracers and diagnostic aids when utilized properly. The objectives of this project are: to ascertain the mechanisms by which radionuclides are deposited in bone and other tissues and cleared therefrom; to seek means of reducing the burden of potentially hazardous radionuclides fixed in the skeleton and elsewhere in the body (radiostrontium and radiocesium receive especial attention); to study by radioisotope labeling methods the behavior of various atomic species normally present in the body (animals and humans), seeking correlations of abnormal behavior with disease states; and to devise tests and techniques of aid in the diagnosis and treatment of such metabolic derangements. Particular emphasis is laid on the metabolism of radionuclides by bone tissue, since several of the products of nuclear fission become fixed tenaciously in the skeleton after entry into the body.

A facility to detect and identify extremely small quantities of radioactive materials in living human beings is maintained. This is used to monitor the radioactive body burdens in infants, children and adults resulting from contamination of the environment by fission products. In addition the Total Body Counter Facility is used in clinical research investigations wherein tracer amounts of radioactive materials are intentionally administered. The doses required in such studies are very small because of the great sensitivity of the equipment.

13. RELATIONSHIP TO OTHER PROJECTS

Related research is being conducted at the Argonne National Laboratory; Brookhaven National Laboratory; University of Rochester AEP; Donner Laboratory; University of California; Los Alamos Scientific Laboratory; University of Utah, AEP; Cornell University.

14. TECHNICAL PROGRESS IN FY 1965

The distributions of fission product zirconium-95 and its daughter niobium-95 among the tissues of pregnant rabbits and their fetuses were determined. Both nuclides cross the placenta following introduction into the maternal blood. For ^{95}Zr, bone is the target tissue of greatest concern in both the maternal and fetal body, concentrations being 5-20 times those in other tissues. For ^{95}Nb, the liver and kidneys receive the greatest burden in the maternal body, but in the fetus liver and bone attain even higher

concentrations. Calculations of radiation dosage to the fetus should therefore not be based on estimates of fetal tissue distributions from data obtained solely from adult animals.

Short term retention of strontium-85 in 23 humans was studied following single intravenous injection. Body burdens were measured in the Total Body Counter Facility at frequent intervals during periods up to several months. Eleven subjects were maintained on metabolic balance regimens in the UCLA Metabolic Service Unit. This group included cases with avid retention of dietary calcium, normals and several with large daily losses of endogenous calcium. ^{85}Sr excretion reflected the state of Ca metabolism. Repetitive external monitoring of knee and tibia divulged no reliable correlation with total body retention and therefore is useless for estimating body burdens. Other methods for estimating human body burdens of the more hazardous ^{90}Sr were reviewed. Available data on ^{85}Sr excretion vs. retention were collected. They suggest that the initial body burden of ^{90}Sr following an acute contamination can be estimated with a standard deviation of ± 38% by multiplying the activity of ^{90}Sr in the complete urine collected during the 7th day following contimination by 46.8.

15. EXPECTED RESULTS IN FY 1966

The results of more than 400 measurements of gamma radioactivity in people residing in the greater Los Angeles area will be summarized and published. This monitoring activity at the Total Body Counter Facility will continue. Current studies of the efficiency of oral absorption and retention of calcium in patients will be pursued, using ^{47}Ca. Other collaborative research efforts with the University Departments of Radiology, Medicine, and Obstetrics will include iron absorption studies, copper metabolism, new methods for measuring bone density and placental transport of calcium, cesium, sodium and potassium.

16. EXPECTED RESULTS IN FY 1967

Measurements of human body burdens of gamma emitting radionuclides will be maintained using the Total Body Counter. This facility will also be utilized for in clinical investigations of disorders in the homeostatic control of body electrolytes and will continue its service function of providing emergency measurements of persons in local and nearby communities who are accidentally exposed to radioisotope contamination. Studies of the metabolism of various radioactive nuclides will continue, using laboratory animals. For example, the kinetics of removal of electrolytes such as calcium, potassium, sodium and chloride from the blood soon after injection will be studied using multiple tracers, circulating blood loops and fast recording on multichannel magnetic tape. Efforts to reduce the detrimental effects of accumulation of radionuclides within the body (particularly radiostrontium and radiocesium) will be pursued.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

1. Contractor: Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. Project Title:

Hematology

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared: April 1965

5. Method of Reporting:
Publications, UCLA Reports
Semi-annual and Final Reports

6. Working Location:
UCLA

7. Person in Charge:
Joseph F. Ross, M. D.

8. Project Term:
From: 1960 To: Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	½	3	4
(b) Other Technical	0	0	0
Total	½	3	4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 13,800	$ 25,300	$ 38,300
(b) Materials & Services	100	4,000	4,000
(c) Indirect Expenses*	(1%) 10,200	(2%) 21,900	(2%) 22,400
Total	$ 24,100	$ 51,200	$ 64,700

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Ionizing Radiation and the Development and Survival of Life. Ross, J.F.: Transactions of the American Clinical and Climatological Association, 77, 1965 (in press).

2. Biological Effects of Ionizing Radiation. Ross, J.F.: Nuclear Medicine, Chapter 6, Blahd, W. H., Ed., McGraw-Hill, N.Y., October 1965.

3. Therapy of Leukemia. Weinstein, I. M. and Ross, J. F.: Medical Science, 14:56-66, 1965.

4. Replacement Rates for Human Tissue from Atmospheric Radiocarbon. Libby, W. F.; Berger, R.; Mead, J. F.; Alexander, G. V. and Ross, J. F.: Science, 146:1170-1172, 1964.

5. Atmospheric Bomb Radiocarbon as a Tracer in Humans. Berger, R.; Libby,W.F.; Mead, J.F.; Alexander, G. V. and Ross, J. F.: Advances in Tracer Methodology, III, Plenum Press, 1965.

12. SCOPE OF THE PROJECT

Radiation exposure, neoplasia, inflammation and other pathological states produce profound modifications in the dynamics of physiological and metabolic processes of the host organism. These changes are of fundamental importance to an understanding of the mechanisms by which disease or other adverse influences produce their effects, and to an approach to prevention or control of these adverse effects. Radioisotope tracer methods are uniquely suited to the investigation of the dynamics of bodily processes and are being used in the study of the effects of abnormal states on these functions.

The research endeavor of this section is concerned with the study of the influences of ionizing radiation, neoplasia and other pathological states on the metabolic and physiologic functions of mammals, including man. The erthropoietic, leucopoetic, and reticuloendothelial systems are being studied employing the radioisotopes of iron, zinc, and carbon as tracers of metabolic processes, and of rates of production and destruction of proteins, lipids and cellular elements.

13. RELATION TO OTHER PROJECTS

1. Studies of effects of radiation, and other pathologic states, on the erythropoietic and reticuloendothelial systems, and on hemoglobin and iron metabolism: Dr. Thomas G. Hennessy, Dr. Arne Schjeide and Dr. George Taplin of this Laboratory; Dr. Clement Finch, University of Washington School of Medicine; Dr. Myron Pollycove, University of California, Berkeley, Donner Laboratory; Dr. G. Cartwright, University of Utah School of Medicine; Dr. Ernest Beutler, City of Hope Medical Center, Duarte, California; Dr. W. Lohmann, University of Arkansas School of Medicine; Dr. L. Wasserman, Mount Sinai Hospital, New York City; Dr. Eugene Cronkite, Brookhaven National Laboratory, Upton, Long Island, New York.

2. Studies of Zinc Metabolisms: Dr. Thomas G. Hennessy and Mr. James OKunewick of this Laboratory; Dr. Herta Spencer, V.A. Hospital; Dr. Ananda Prasad, Wayne University School of Medicine.

3. Studies of C^{14} turnover in human tissues are being conducted in collaboration with Dr. James Mead and Mr. George Alexander of this Laboratory and Dr. Willard F. Libby and Dr. Rainer Berger of the Department of Chemistry and Institute of Geophysics, UCLA.

14. TECHNICAL PROGRESS IN FY 1965

1. Studies of the influence of inflammatory processes on radioiron metabolism have been completed and are in final manuscript form. These studies have demonstrated a marked depressant effect of inflammatory processes on the reutilization for hemoglobin synthesis of iron released from senescent erythrocytes, although acute inflammatory processes do not significantly effect the utilization of injected transferrin bound iron for hemoglobin synthesis. It is hypothesized that inflammatory processes interfere with the degradation of hemoglobin iron by the reticulo-endothelial system, and thus interfere with the normal cycle of reutilization of iron released from the destruction of erythrocytes.

2. Studies of the metabolism of Zinc 65 in human subjects with neoplastic diseases have been completed and are in process of final publication. These studies have demonstrated different turnover rates for zinc in various tissues, and a total body turnover rate with an average of half time of 365 days. Zinc rapidly enters erythrocytes, but is not firmly bound, since it leaves biologically labelled red cells at a rapid rate. Leucocytes rapidly take up intravenously injected Zinc 65, and the concentration in these cells increases after plasma Zinc 65 levels have decreased. Zinc 65 is slowly discharged from leucocytes, but persists for at least 30 days after injection.

3. The 100% increase in atmospheric Carbon 14 produced as a consequence of testing of nuclear weapons has made possible studies of the incorporation of C^{14} into human tissues. These studies have been conducted in collaboration with Dr. James Mead and Mr. George Alexander of this Laboratory and Dr. Willard F. Libby and Dr. Rainer Berger of the UCLA Department of Chemistry and Institute of Geophysics. We have shown that C^{14} derived from the atmosphere, probably through the food chain, but possibly also from inhalation of C^{14}, is incorporated into proteins and lipids of the central nervous system in approximately equal concentrations - a most interesting finding, since the metabolism of brain lipids has been considered to be relatively inactive.

   Incorporation of C^{14} into hepatic and myocardial proteins also occurred in significant and similar amounts. The cartilage of elderly male subjects did not show incorporation of C^{14} in amounts in excess of background levels, confirming the "non labile" state of this tissue.

   Plasma and erythrocyte proteins showed incorporation of C^{14} in differing amounts, presumably as a reflection of different turnover rates.

4. The responsible investigator of this section has had the major responsibility for the development and direction of the educational program in biophysics, nuclear medicine, and radiation biology. During 1964-65 this program has come to fruition and the educational and training potentialities of the Laboratory of Nuclear Medicine and Radiation Biology have been realized. Twenty-nine predoctoral students are enrolled in the Department, and four Ph.D. degrees and two masters degrees were awarded during this year. Seventeen post-doctoral trainees from the U. S. and 10 foreign countries are studying under the auspices of the Department.

Six medical students and ten undergraduate college students served as research assistants to Laboratory staff during the summer months, and twelve undergraduate and four graduate students were employed as technicians or assistants during the school year.

There has been a nine fold increase in instructional activity during the past years. A unique graduate course in environmental radiation has been introduced and has been very successful.

15. EXPECTED RESULTS IN 1966

1. Studies of the effects of ionizing radiation and inflammatory processes on iron metabolism, on erythropoiesis, on erythrocytic survival and on reticulo-endothelial system function will be extended to human subjects and continued in other mammals. We expect to demonstrate that the anemia observed in these states is attributable in part to reticulo-endothelial system dysfunction.

2. The importance of Zinc 65 as an environmental contaminant resulting from operation of nuclear reactors is widely recognized. This isotope is incorporated into certain substances which may serve as human food stuffs. We propose to study the metabolism of Zinc 65 contained in such material in human subjects, employing the total body counter. Additionally we will investigate the metabolism of zinc and zinc containing enzymes in human erythrocytes and tissues with the expectation of demonstrating an active metabolic turnover of such substances (e.g. erythrocyte carbonic anhydrase). Modification of turnover rates by disease processes, and by therapeutic measures will be studied.

3. Atmospheric Carbon 14 equilibration with human tissues will be studied, and related to temporal and geographic factors. Arrangements have been made to obtain tissue samples from geographic areas of the earth in which the atmospheric C14 content is markedly different from that prevailing in Los Angeles, and changes of tissue concentrations of C14 will be correlated with changes in atmospheric C14.

   Variations in C14 content of human tissues with variations in the age of the subject will be studied, and the observations indicating that central nervous system lipids have a significant metabolic turnover will be expanded and refined. Additionally, attempts will be made to study metabolic processes of other tissues employing atmospheric derived C14. These studies will be conducted in collaboration with Drs. Berger, Libby and Mead and Mr. Alexander.

4. The educational and training programs will be advanced in accordance with contractual obligations.

16. EXPECTED RESULTS IN 1967

1. Employing radioactive tracer techniques, investigations will be made of the metabolic and physiologic processes in human subjects subjected to radiation or suffering from various pathologic abnormalities. These studies will be oriented primarily toward determining the effects of radiation on the reticulo-endothelial, the erythropoietic and the leucopoietic systems.

2. Studies of Zinc 65 metabolism, and metal containing enzymes, and of the effects of ionizing radiation on their concentration and function will be expanded.

3. Studies of the metabolism of environmental Carbon 14 by human subjects will be continued, with the intent of establishing turnover rates of this material in humans. The possibility of employing C^{14} derived from environmental sources to assess metabolic and physiologic functions in human subjects will be thoroughly explored.

SCHEDULE 189
ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

1. **Contractor:** Laboratory of Nuclear Medicine and Radiation Biology
University of California, Los Angeles

Contract No.: AT(04-1)GEN-12

2. **Project Title:**
Mammalian Radiobiology

3. **AEC Budget Activity No.:**
06-10-00

4. **Date Prepared:**
April - 1965

5. **Method of Reporting:**
Publications, UCLA Reports
Semi-annual and Final Reports

6. **Working Location:**
UCLA

7. **Person in Charge:**
T. G. Hennessy, M. D.

8. **Project Term:**
From: 1954 **To:** Continuing

9. Man Years	FY 1965	FY 1966	FY 1967
(a) Scientific	3	3	4
(b) Other Tech.	0	0	0
Total	3	3	4

10. Costs	FY 1965	FY 1966	FY 1967
(a) Direct Salaries	$ 27,300	$ 33,000	$ 38,300
(b) Materials & Services	3,500	4,000	4,000
(c) Indirect Expenses*	(2%) 20,400	(3%) 33,000	(2%) 22,400
Total	$ 51,200	$ 70,000	$ 64,700

* Total indirect expense of the Contract pro-rated among individual projects on the basis of the percentage of total direct salary expense represented by the particular project.

11. PUBLICATIONS DURING FY 1965

1. Early Response of Pinus thunbergii to acute γ-Irradiation. J.P. OKunewick, S.E. Herrick, and E.N. Carlsen. Nature 204, 394-5 (1964).

2. Ultracentrifugal Assay of Zinc Binding in the Proteins of Chicken Eggs and Serum. J.P. OKunewick, O.A. Schjeide and G. Glancy. Biophysical Journal 5, 35 (1965) (Abstracts).

3. Studies of Red Cell Carbonic Anhydrase following Lethal X-Irradition. J.P. OKunewick, G. Glancy, and T.G. Hennessy. Radiation Research 24 (1965) (Abstracts).

12. SCOPE OF THE PROJECT

The study of blood and of hematopoiesis has provided much important information both as regard to the extent of injury following exposure to ionizing radiation and also in pathological conditions not normally associated with radiation damage. In this regard radioiron uptake has proven particularly valuable in assaying erythropoetic damage and recuperability following radiation injury. In the study of the mechanism of erythropoiesis and in the stimulation of erythropoietic action following radiation radioiron is also proving to be of particular use and present studies are being concentrated along these lines.

The study of radiozinc binding in the blood has led to interesting applications of this isotope and of C^{14} in the investigation of the response of the CO_2 transport regulating enzyme, carbonic anhydrase, to x-irradiation in vivo. Work along these lines is proceeding with the intent of determining whether or not alterations in the CO_2 transport mechanism of the blood may be involved in radiation mortality in mammals.

13. RELATIONSHIP TO OTHER PROJECTS

Similar work on Zn^{65} metabolism is being carried out at Hanford Atomic Products Operation, Richland, Washington, and at Los Alamos Scientific Laboratory.

Studies on Fe^{59} are being carried out at: Lawrence Radiation Laboratory, University of California; Argonne Cancer Hospital, Chicago, Illinois; University of Buffalo, Buffalo, New York; and Naval Radiological Defense Laboratory, San Francisco, California.

14. TECHNICAL PROGRESS IN FY 1965

Studies in erythropoiesis have proceeded along promising lines. The association of the erythropoietic hormone with cerebellar hemangioblastomas has been investigated and these have been found to contain large amounts of the hormone. Studies on radiozinc turnover in relation to carbonic anhydrase have indicated the possibility of a slow but consistent exchange of the enzyme in vivo. Radiation studies of the enzyme involving C^{14} uptake indicate that the enzyme may still be produced in vivo after irradiation but at a lowered rate.

15. EXPECTED RESULTS IN FY 1966

Further investigations involving the use of radio-iron and erythropoietin will be carried out. Installation of a high-altitude chamber is expected to facilitate and accelerate these studies. In addition the effects of radiation on other blood systems not heretofore extensively examined will be investigated.

16. EXPECTED RESULTS IN FY 1967

Investigation will continue along the same lines depending upon the results of current investigation without any major change in scope or level of the program.