UCLA

UNIVERSITY OF CALIFORNIA, LOS ANGELES

SCHOOL OF MEDICINE

RESEARCH AND DEVELOPMENT PROGRAM
FISCAL YEAR 1966

APRIL 1964

DEPARTMENT OF BIOPHYSICS AND NUCLEAR MEDICINE

LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY

CONTRACT NO. AT (04-1) GEN-12

DISCLAIMER

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 1966

APRIL 1964

MASTER

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LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY
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 1965 and FY 1966

In general, the overall direction and level of the Laboratory's programs remain unchanged, but with moderate expansions in some projects and slight shifts of emphasis among others during FY 1965 and FY 1966. In Molecular and Cellular Level Studies, the current moderate expansion is expected to continue with 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 acid coding systems and related enzyme studies. In the Terrestrial and Fresh Water Ecology category, studies in the ecological characterizations of the Nevada Test Site, balance studies of radionuclides in an active dairy farm in Utah, and various studies of controlled, continuous low-level radiation of natural populations and vegetation in the Rock Valley area are expected to reach their maximum activity during FY 1966 and the three years immediately following. In the Atmospheric Radioactivity and Fallout category, the shift in emphasis from collection and physical characterization of fallout has resulted in an expansion of programs dealing with the development of mathematical models for isotopes in the food chain and the design of experiments to confirm the accuracy of these models, as well as field studies to determine rates and patterns of transfer of fallout radionuclides from the atmosphere to soil plants, and animals. The evaluation of fallout data previously collected during large scale field tests will continue but at a decreasing level of effort. In Selected Beneficial Applications the current increased level of effort in studies for the development of techniques for the use of radioisotopes in clinical diagnosis and elucidation of metabolic function will be continued and expanded. The Total Body Counter will play an increasingly important role in these studies. It is anticipated that other programs in the categories of Radiation Effects-General, Combating Detrimental Effects of Radiation, Radiological and Health Physics, Radiation Instruments, Chemical Toxicity, and Cancer Research will be continued at approximately their current level of effort.

General Comments on Program Costs:

The composition of costs and staffing for FY 1964, FY 1965, and FY 1966 are summarized below for the entire Biology and Medicine Program by major categories of expense.

	FY 1964	FY 1965	FY 1966
I. Costs: (Shown as Thousands)			
Salaries and Burden (Direct)	$1,258.8	$1,492.7	$1,617.0
Supplies and General Expense	235.7	215.6	250.4
Indirect Costs	1,055.5	1,110.4	1,134.1
Total Operating Costs	$2,550.0	$2,818.7	$3,001.5
II. Manpower: (Shown as Full Time Equivalence)			
Direct Man Years	147¼	152½	160
Direct Scientific Man Years	129½	134¾	141¾

III. Cost/Manpower Data:

Cost/Direct Man Years	$ 17.3	$ 18.5	$ 18.8
Cost/Direct Scientific Man Years	$ 19.7	$ 20.9	$ 21.2

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 pro-rating 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 pro-ration 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.

Composition of Indirect Expense:	FY 1964	FY 1965	FY 1966
I. Manpower (Shown as Full Time Equivalence):			
Administrative Services	42 ½	41 ½	41 ½
Technical Services	18 ½	20	20
Building Maintenance	8 ½	8 ½	9
Total Indirect Personnel	69 ½	69 ½	70 ½
II. Costs: (Shown in Thousands)			
Administrative Services	$ 405.5	$ 416.8	$ 425.1
Technical Services	175.6	213.6	221.5
Building Maintenance	72.5	78.0	85.5
Occupancy Charge	200.0	200.0	200.0
Utilities	67.5	67.5	67.5
U.C. Management Allowance	75.0	75.0	75.0
U.C. Accounting & Purchasing Services	36.1	36.1	36.1
Miscellaneous (Security, Laundry, Postage, General Supplies, etc.)	23.3	23.4	23.4
Total Indirect Costs	$1,055.5	$1,110.4	$1,134.1

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 1964 COSTS	FY 1964 MAN YRS	FY 1965 COSTS	FY 1965 MAN YRS	FY 1966 COSTS	FY 1966 MAN YRS
06-01-01	Radiation Effects-General						
	Medical Physics Problems (B. Cassen)	$ 61.3	3¼	$ 59.0	3¼	$ 60.6	3¼
	Effect of Radiation on the Nervous System (T. J. Haley)	79.3	5	102.9	5½	106.1	5½
	Post-Irradiation Gastrointestinal Function (L.E. Detrick)	51.0	3	56.4	3	59.1	3
	Late Effects Radiobiology (L. Bennett and B. Lamson)	30.7	3¾	34.7	3	34.9	3
	ACTIVITY TOTAL	$ 222.3	15	$ 253.0	14¾	$ 260.7	14¾
06-03-00	Combating Detrimental Effects of Radiation						
	Radiation Therapeutics (J. Leitch)	$ 69.0	3½	$ 64.9	3½	$ 67.6	3½
	ACTIVITY TOTAL	$ 69.0	3½	$ 64.9	3½	$ 67.6	3½
06-04-00	Molecular & Cellular Level Studies						
	Enzyme Chemistry (I. Harary)	$ 95.0	5½	$ 86.0	5	$ 90.9	5
	Macromolecular Chemistry (N. Simmons)	52.5	3½	52.6	2½	89.5	4½
	General Metabolism (J. F. Mead)	76.1	5	86.1	5	88.0	5
	Organic Chemistry (D. R. Howton)	49.7	2½	54.6	2½	55.1	2½
	Tracer Synthesis (J. C. Nevenzel)	45.9	2½	49.3	2½	50.9	2½
	Pathology (T. G. Hennessy - Acting)	51.1	4	52.1	3½	53.7	3½
	Chemical Radiobiology I (L. S. Myers)	146.7	9	145.3	9	150.8	9
	Chemical Radiobiology II (J. F. Ward)	32.5	2	33.8	2	35.1	2
	Metabolic Radiobiology (O. A. Schjeide)	78.9	4½	107.3	5½	101.5	5½
	Cellular Radiobiology (N. de T. Whittaker)	23.5	1	53.2	3	66.2	4
	Physical Radiobiology (E. H. Strickland)	23.6	1	57.4	3	63.8	3½
	ACTIVITY TOTAL	$ 675.5	40½	$ 777.7	43½	$ 845.5	47
06-05-01	Terrestrial & Fresh Water Ecology						
	Soil Factors (H. Nishita)	$ 65.3	3¾	$ 75.9	4	$ 77.7	4
	Plant Factors (E. M. Romney)	72.8	4½	79.8	4½	71.0	4½
	Environmental Decay (H. Hawthorne)	113.5	6	116.2	6	119.3	6
	Plant Physiology (W. A. Rhoads)	42.2	2½	55.1	3	58.5	3
	Radiation Ecology-Mammalian Irradiation (N. French)	91.5	5	110.3	6	118.7	6½
	Ecology of the Nevada Test Site (J. Beatley)	73.9	4¾	86.0	5	88.9	5
	Radiation Ecology-Lizard Irradiation (F. Turner)	35.7	2½	50.5	2½	53.8	2½
	Radiation Ecology-Plant Irradiation (W. Martin)	34.5	2	51.6	2½	53.7	2½
	Chemical Problems-Ecology (R. Wood - Acting)	87.1	5	92.7	5	98.4	5
	ACTIVITY TOTAL	$ 616.5	36	$ 718.1	38½	$ 740.0	38¾

06-05-03	Atmospheric Radioactivity and Fallout						
	Chemical Problems-Fallout Studies (R. Wood-Acting)	$ 80.1	5	$ 83.9	5	$ 88.2	5
	Nuclear Events-Biological Studies (F. Turner)	77.2	3½	82.7	3½	100.6	4¾
	Environmental Assessments-Plant Studies (W. Martin)	93.6	5	83.5	5	86.8	5
	Physical & Radiological Characteristics of Fallout (K.Larson)	48.5	2	52.2	2	61.0	3
	ACTIVITY TOTAL	$ 299.4	15½	$ 302.3	15½	$ 336.6	17¾
06-06-01	Radiological & Health Physics						
	Radiation Dosimetry (G. V. Taplin)	$ 45.1	2¼	$ 30.0	1¼	$ 30.2	1¼
	ACTIVITY TOTAL	$ 45.1	2¼	$ 30.0	1¼	$ 30.2	1¼
06-06-02	Radiation Instruments						
	Medical Physics Instrumentation (B. Cassen)	$ 75.8	3¼	$ 62.5	3¼	$ 63.0	3¼
	ACTIVITY TOTAL	$ 75.8	3¼	$ 62.5	3¼	$ 63.0	3¼
06-07-00	Chemical Toxicity						
	Chemical Toxicity of Rare Earths (T. Haley)	$ 48.3	2½	$ 53.5	2½	$ 55.8	2½
	ACTIVITY TOTAL	$ 48.3	2½	$ 53.5	2½	$ 55.8	2½
06-09-00	Cancer Research						
	Biological Studies of Leukemia (E. F. Hays)	$ 71.8	4½	$ 61.6	4¼	$ 62.1	4¼
	ACTIVITY TOTAL	$ 71.8	4½	$ 61.6	4¼	$ 62.1	4¼
06-10-00	Selected Beneficial Applications						
	Clinical Nuclear Medicine (G. V. Taplin)	$ 127.4	6	$ 147.3	7¼	$ 139.0	7¼
	Basic Nuclear Medicine (G. V. Taplin)	47.7	3¾	62.0	4½	81.9	5
	Hemodynamics (G. V. Taplin)	28.9	1¾	36.6	1½	38.9	1½
	Nuclide Metabolism (N. MacDonald)	103.3	6¼	108.5	5¾	117.1	6¼
	Hematology (J. F. Ross - Acting)	68.4	3¼	82.4	3½	86.4	3½
	Mammalian Radiobiology (T. G. Hennessy)	50.6	3¼	58.3	3	76.7	3½
	ACTIVITY TOTAL	$ 426.3	24¼	$ 495.1	25½	$ 540.0	27
	Total 06-00-00 Program	$2,550.0	147¼	$2,818.7	152½	$3,001.5	160

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 - 1964

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: 1955 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2 1/2	2 1/2	2 1/2
(b) Other Tech.	3/4	3/4	3/4
Total	3 1/4	3 1/4	3 1/4

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 30,000	$ 34,100	$ 35,200
(b) Materials & Services	1,000	2,700	2,700
(c) Indirect Expenses* (3%)	30,300	(2%) 22,200	(2%) 22,700
Total	$ 61,300	$ 59,000	$ 60,600

* 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 1964

Two papers were read at the Milwaukee Meeting of the Radiation Research Society. They are (1) The Effect of Total Body Irradiation on the Size Distribution of Circulating Lymphocytes - by R.D. Neff and B. Cassen, and (2) Stimulation of Extracorporeal Blood Irradiation by the Localized Tail Irradiation of Mice- by B. Cassen, M. Moody and R. Neff.

12. SCOPE OF PROJECT

In this project, much of our effort devoted to the use of new techniques and methods in the study of the effects of acute radiation on circulating lympocytes. The physiology and kinetics of lymphocytes are of great complexity. There is strong evidence that lymphocytes recirculate by way of the lymphatic system through the thoracic duct and back into the blood. They also leave the blood and pass into most body tissues where, as is well known, they exist in large numbers. Their ultimate history and fate are still unknown. There is increasing evidence that under antigenic stimulation even in vitro they can transform into other imunologically functioning cell types. In an initial attempt to help unravel this complex situation we have been studying some of the factors involved in the rapid disappearance of circulating lymphocytes after total body irradiation and in the rebound phase which occurs after the minimum cell count. One of the methods we have developed for this study consists in employing a multichannel analyzer in conjunction with a Coulter counter. This makes possible the determination of a complete size distribution spectrum with high resolution. The following indicates some of the potentialities of this development. A determination of size distribution of lymphocytes, separated from a one ml blood sample, can be made in about 2 minutes. Accordingly, the lymphocyte size spectrum of a single animal can be followed sequentially in time after irradiation, thereby greatly reducing the statistical uncertainties of using a new animal for each time after irradiation. It has been found possible, by the use of the logarithmic display feature of the multichannel analyzer, to resolve the statistical occurrence of extra large lymphocytes which might be present only in one or two per 10,000 cells. By this means we expect to be able to determine the relative occurrence of binucleate lymphocytes before and after irradiation.

Also, these methods have confirmed results obtained previously that radiation causes small lymphocytes to decrease in number more rapidly than large lymphocytes, although the latter are also reduced in absolute number. There are several hypotheses that would explain this effect. Further experiments are being undertaken in an attempt to exclude or prove certain possible explanations. One theory proposes that large lymphocytes, which are known to be capable of mitotic division comprise a stem cell system for the generation of medium and small lymphocytes. To help in elucidation of the effect of radiation on large lymphocytes we are growing separated lymphocyte cell cultures from an animal just prior to and immediately after an acute dose of radiation. The comparative yield of mitotic figures observed after 72 hours in cultures stimulated by phytohemagglutinin and finally treated with colchicine indicates the direct effect of the total body irradiation on the large lymphocytes.

In order to gain further information on experimentally detectable classes of lymphocytes, we have developed and are using an electrophoretic cell separator which, amongst other uses, can be used to fractionate lymphocyte groups into fractions of different zeta potential. The cells remain viable and can be studied in cell cultures. In preliminary tests the electrophoretic fractions have been found to have different size distributions. It is very likely that the zeta potential of a cell is related to its metabolic activity and this in turn is correlated with its size. The data is being analyzed to explore its significance.

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

14. TECHNICAL PROGRESS IN FY 1964

By the use of techniques and methods indicated in Section 12 above, the following results were obtained or indicated.

(a) It was confirmed that after acute irradiation of rabbits at various dose levels in the neighborhood of 100 to 300 rads, small lymphocytes decrease in number considerably faster than large lymphocytes, although the latter also decrease in absolute number. The high resolution quality of the size spectra now being obtained enables much better resolution of detail in the size distribution. Definite maxima and minima are observable and change after irradiation. Their significance is being studied.

(b) By means of the logarithmic display feature of the multichannel analyzer it appears that the occurrence of large lymphocytes, although only present in one or two out of two thousand cells, can be detected with good statistical significance. The number of such cells increases after irradiation. We are trying to determine whether or not they can be identified as binucleate lymphocytes or as extra large lymphocytes arising from antigenic or other stimulation.

(c) By the procedure of growing cell cultures from separated lymphocytes from a rabbit before and after *in vivo* irradiation, it has been shown that the absolute number of mitotic figures is reduced in the cultures prepared from the animal after irradiation. However, the total number of lymphocytes (small and medium) is reduced even more in proportion.

(d) Preliminary results obtained with the electrophoretic cell separator indicated in Section 12 above show that the viability of fractionated lymphocytes can be maintained through the fractionation procedure. The size distribution of fractionated lymphocytes is changed in the various fractions. The significance of these observations is being analyzed. It is probably related to a correlation of zeta potential and size mediated via metabolic activity.

15. EXPECTED RESULTS IN FY 1965

It is expected that thorough and systematic measurements can be made of changes in lymphocyte size distributions after *in vivo* total body irradiation through the complete time sequence of injury, rebound and recovery. It is expected that similar measurements can be obtained on electrophoretic fractions of these cells. The data will be carefully analyzed for the light it will throw on the physiology and kinetics of lymphocytes, and their response to exposure to ionizing radiation.

It is expected that a relatively simple and inexpensive technique can be evolved to determine the incidence of large lymphocytes (possibly binucleates) after low doses of radiation.

16. EXPECTED RESULTS IN FY 1966

It is expected in FY 1966 that further development of techniques and methods for measuring properties of lymphocytes will help further in unravelling their complex physiology and radiation biology. It is expected that a program will be initiated of determining the effects *in vitro* and *in vivo* of various agents such as certain pharmacologically active agents, polypeptides, 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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	4	4 ½	4 ½
(b) Other Tech.	1	1	1
Total	5	5 ½	5 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 45,500	$ 55,000	$ 55,000
(b) Materials & Services	3,500	3,500	5,700
(c) Indirect Expenses* (3%)	30,300	(4%) 44,400	(4%) 45,400
Total	$ 79,300	$102,900	$ 106,100

* 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 1964

1. Chemical Protection Against Three Types of Radiation Death (Hematopoietic, Gastrointestinal and the Central Nervous System), Haley, T.J., Nuclear Hematology 2, 3-7 (1963).

2. Protection Chimique Contra Trois Types de Radiations Lethales - A Sovoir Lesions Hematopoietiques, Gastro-Intestinales et Nervesueses, Haley, T.J., Journees d'Etude Sur Les Effets Physiologiques des Radiations Ionisantes, Oct. 1, 1962, pp. 1-14.

3. Aspects Nouveaux Dans Le Developpement des Substances Radio-Protectrices, Journees d'Etude Sur Les Effets Physiologiques des Radiations Ionisantes, Haley, T.J.,Oct. 1, 1962, pp. 31-37.

4. Failure of Quinoxaline-1,4-di-N-Oxide to Increase the Survival Time in Irradiated Autologous Marrow Infused Dogs, Trumbull, W.E., Kaeakami, J.A., Cannon, J. and Haley, T.J., J. Surg. Res. 4, 103 (1964).

5. Effects of Ionizing Radiation on the Bioelectrical Activity of the Central Nervous System, Gangloff, H. and Haley, T.J., Jour. Neurology, in press.

6. Response of the Adrenal Cortex to Radiation, Hameed, J.L. and Haley, T.J., Fed. Proc. 23, 571 (1964).

7. Effect of X-Radiation on Mineral Content of Selected Brain Areas of Rat Brain, Haley, T.J., Swimmer, D. and Komesu, N., VI Intl. Cong. of Brain Biochemistry, in press.

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 precursor-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 1964

Studies of the brain mineral element composition using the X-ray fluorescence method indicated that phosphorus levels were consistently low in the cerebral cortex 2, 4 and 7 hours after radiation dosages ranging from 600 to 1200 r. Such results indicate an increased utilization of high energy phosphate by the brain following irradiation. These results correlate with our previous observation of increased brain excitability early in the radiation syndrome. Determinations of blood and adrenal gland cortico-steroids show two peaks in activity, one at 2.5 hours and the other 48 to 72 hours after irradiation. Hypophysectomy prevents these changes in the corticoid levels in the blood and in the gland. Moreover the use of the beta-hydroxylase inhibitor indicates that this enzyme is not damaged by irradiation. An intact pituitary adrenal axis is essential for the release of these materials in response to ionizing radiation. Continuing studies of bar pressing activity in trained rats have indicated that the results obtained do not differ significantly in overall effect when a short acting drug is given or when a long acting one is given. The major difference is related to the recovery period which can be prolonged for a period of 3 weeks with a single dose of reserpine or 3 days with tetrabenazine. Inasmuch as these drugs release biogenic amines and the electrode is implanted in the area of their greatest concentration, we believe that these materials must have some correlation with the delayed functional recovery in radiation injury.

15. EXPECTED RESULTS IN FY 1965

Upon the basis of previous work, perfusion of the cat brain via the ventricular system coupled with analysis of the effluent will enable an estimate to be made of tissue release of neurohormones under the influence of both drugs and irradiation. Concomitant analysis of selected brain areas should give information on the rate and type of release of neurohormones by irradiation. Correlation of these results of measurements of electrical activity may give an insight into the inhibitory or stimulatory effects of irradiation on selected brain areas.

16. EXPECTED RESULTS IN FY 1966

Upon the basis of results obtained in the acute whole-body irradiation studies in the unanesthetized cat, chronic low radiation dose studies will be continued. 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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	3	3	3
(b) Other Tech.			
Total	3	3	3

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 28,600	$ 31,900	$ 34,100
(b) Materials & Services	2,300	2,300	2,300
(c) Indirect Expenses* (2%)	20,100 (2%)	22,200 (2%)	22,700
Total	$ 51,000	$ 56,400	$ 59,100

* 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 1964

1. Increased Intestinal Elimination of N'-methylnicotinamide in Irradiated Rats, Detrick, L.E., Upham, H.C., Springsteen, R. and Haley, T.J. Int. Jour. Rad. Biol. 7, 161-166 (1963)

2. Pyridoxine Absorption from Isolated Irradiated Rat Intestine in Situ. Detrick, L.E., Upham, H.C., Springsteen, R.W., McCandless, R.G. and Haley, T.J., Radiation Research, in press.

3. Postirradiation Intestinal Absorption of L-Methionine in Rats, Detrick, L.E. and Upham, H.C., Fed. Proc., in press.

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 organisms 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 1964

Electron Microscopy demonstrated that control rats absorbed fed lipids across the external microvillar membrane, but absorption by pinocytosis was lacking. Droplets appeared, grew and were transported within agranular endoplasmic reticulum, but the controversial extent of this lipid transport pathway was not resolved. An occasional large cytoplasmic lipid droplet was observed within control rat intestinal epithelium. Irradiation increased microvillar lipid granules, lipids within the reticulum and Golgi apparatus, absorbed cytoplasmic lipid material and large cytoplasmic droplets. Large cytoplasmic droplets appeared on day 3, persisted on day 17 and were normal on day 28. They developed within otherwise normal appearing epithelial cells and grew by canalization with

and subsequent absorption of smaller cytoplasmic droplets. Their bud-like and ruffed border appeared to indicate that growth and lytic processes progress simultaneously. Static lipid morphology failed to distinguish the dynamics of lipid absorption. Postirradiation intestinal L'methionine absorption trends were similar to but not identical with those reported for thiamine and pyridoxine. All were recovered on day 28. Histological and ultrastructural study in thiamine and pyridoxine absorption rat intestine also showed recovery at that time. However, L'methionine absorption was depressed to an even lower level by a second 525 r exposure on day 28 and pre-treatment with AET prior to the second irradiation decreased the double irradiated deaths from 60% to 11%. Neither recovery of the animal nor recovery of intestinal function had returned to its original condition by day 28, and there was a residual increased sensitivity to irradiation. However, intestinal ultrastructure that appeared normal on day 28 may have contained changes too subtle to have been previously recognized.

15. EXPECTED RESULTS IN FY 1965

Lipid absorption ultrastructure will receive further consideration in an attempt to resolve the controversial problem of microvillar versus pinocytic absorption or both. Intestinal L'methionine absorption curves will be correlated with ultrastructural morphology from non-perfused intestinal segments of the same animals. The investigation will be repeated using tryptophan, double irradiation and AET treatment, but ultrastructural studies will be omitted. The period required for intestinal recovery following a single 525 r radiation exposure remains to be determined.

16. EXPECTED RESULTS IN FY 1966

Plans have been made to expand the postirradiation study of intestinal lipid absorption morphology through the use of radiotagging and/or radioautographs. The N'methylnicotinamide study already reported implicated radiation degradation products released from the body organs as a possible source of injury that led to focal epithelial degeneration on days 10-17. Such products were increased in the urine and also eliminated in the lumen. The problem would not be complete without employing body shielding and local intestinal 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:
Late Effects - Radiobiology

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

4. Date Prepared:
April - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
L.R. Bennett and B.G. Lamson

8. Project Term:
From: 1950 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2 1/4	2	2
(b) Other Tech.	1 1/2	1	1
Total	3 3/4	3	3

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 16,900	$ 19,800	$ 19,800
(b) Materials & Services	3,800	3,800	3,800
(c) Indirect Expenses*	(1%) 10,000	(1%) 11,100	(1%) 11,300
Total	$ 30,700	$ 34,700	$ 34,900

* 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

(1) Effect of Single and Divided Doses of X-Irradiation Upon Longevity of Rats. B. G. Lamson, M. S. Billings, J. J. Gambino, and L. R. Bennett. Radiation Research 18: 255-264, 1963.

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 property define the conditons 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); Kohn (UCSF); Anderson (UC-D); Kaplan (Stanford); Vogel (Argonne); Conard, Bond (Brookhaven); Cole (USNRDL); Upton (Oak Ridge); Gowen (Univ. of Iowa); Boone (Los Alamos); and Bustad (Hanford).

14. TECHNICAL PROGRESS IN FY 1964

Statistical analysis and preparation for publication of completed experiments continued during the year.

A study of the influence of anoxia at the time of irradiation on the incidence and persistence of chromosome aberrations in rat bone marrow and liver was begun, and is partially completed.

Because of the extensive use in clinical medicine of the radioisotope Hg^{203}, which concentrates in the kidney, the possibility of latent injury must be considered seriously. Studies previously reported from this laboratory have demonstrated that latent renal injury occurs at doses of a few hundred rads, indicating that the kidney is far more radiosensitive than is commonly believed. Since a decline in renal function in the human correlates closely with age, and renal failure or hypertension are frequent causes of death, it is reasonable that agents which may reduce kidney reserve should be critically evaluated before being extensively used in clinical medicine. Experiments have therefore been set up to evaluate possible injury to the rat kidney from doses of Hg^{203} in the diagnostic range, using the NaCl toxicity test. This test,

which was developed in this Laboratory, is probably the most sensitive test for latent radiation injury in the irradiated rat.

15. EXPECTED RESULTS IN FY 1965

Results of previous experiments on partial body irradiation will be reported. The first studies on chromosome aberrations and aging, as influenced by anoxia at the time of irradiation, will be completed. The toxicity study on Hg^{203} should also be completed.

16. EXPECTED RESULTS IN FY 1966

Work will continue to revolve around the basic problem of the nature of the life shortening effect of irradiation. This will be approached by life span studies of the incidence of disease and age specific organ function in the locally and totally irradiated animal. The late effects of selective irradiation of the liver, kidney, and extremities will be further investigated. Longevity is the variable of prime interest in this program and will be used as the end point measure of radiation effect. This approach requires 3 to 4 years for each experiment. A search will continue for physiologic measures that correlate well with ultimate longevity and, hopefully, will provide an earlier measure of delayed radiation effects. The demonstration in this laboratory that at least one metabolic aberration, i.e., enhanced salt toxicity, can be measured in post-irradiated rats during the disease-free latent period is a stimulus to continue the effort to define the late effects syndrome and longevity in biochemical and physiologic terms. Carbon tetrachloride and other forms of biochemical stress will be studied as a means of bringing out latent injury.

If current studies on chromosome aberrations appear to be significant, they will be continued and correlated with the biochemical 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:
Radiation Therapeutics

3. AEC Budget Activity No.:	4. Date Prepared:
06-03-00	April - 1964
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: James L. Leitch	8. Project Term: From: 1958 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	3 ½	3 ½	3 ½
(b) Other Tech.			
Total	3 ½	3 ½	3 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 32,600	$ 36,300	$ 38,500
(b) Materials & Services	5,900	6,400	6,400
(c) Indirect Expenses* (3%)	30,500	(2%) 22,200	(2%) 22,700
Total	$ 69,000	$ 64,900	$ 67,600

* 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 1964

None

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. RELATED 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 1964

To extend the information available on the relationship between total dose, dose rate, type of ionizing radiation used and efficiency of AET and 5-HT as so-called protective agents, mice have been irradiated with the 10,000 curie Co-60 source. It has been found that the standard pretreatment dose of 10μM AET + 1μM 5-HT showed protection when given 5-10 minutes prior to onset of irradiation at total doses of 900r and 1050 r delivered at 400 or 1600 r/min. However, little or no protection was seen when the total dose was increased to 1200 or 1350 r. Increasing the time between drug injection and onset of irradiation had an effect comparable to that of increasing the total dose of irradiation. Using the Co-60 gamma rays it has been shown that at dose rates of 80-1600 r/min, protection from AET and 5-HT can be demonstrated in mice only if the total time between injection of drug and completion of the radiation exposure does not exceed 40 to 60 minutes.

Since no data are available on what may be called instantaneous exposures this was approximated in several preliminary experiments involving mice exposed in the center of the Co-60 source (rate = 100,000 r/min.) For total doses of 5000, 9000, and 17,000 r (exposures of 2,4, and 8 seconds respectively), all mice died showing general ataxia within three to five days whether pretreated with AET + 5-HT or untreated. Little or no difference in survival times were observed in the above dose range. Mice receiving only a one second exposure (3000 r) died in 4-6 days if untreated but in 4-9 days if pretreated. So far as is known the experiments are the first carried out at this high rate of gamma-irradiation. The results did not include convulsions as a sequelae of high intensity irradiation to the central nervous system as was initially postulated.

A correction for the 10,000 curie Co-60 source was determined to account for the irradiation received during the period that the source is rising and falling (this period is not included in the control clock timing). As of Sept. 23, 1963, values were obtained as follows: (a) at center, 1021 r; (b) 28 cm from center, 60 r; and (c) 142 cm from center, 6-7 r. On this same date the center dose rate in r is calculated as follows:

$$\text{Total dose (r)} = 1000\ r + 2000\ r\ (\text{time in seconds})$$

This correction must be included in all future calculations of radiation dosage using the Cobalt-60 source.

The computer program being developed for the analysis of post-irradiation survival times has been delayed by a transfer to another computing facility on the U.C.L.A. Campus.

15. EXPECTED RESULTS IN FY 1965

Evaluation of the relationships between dose rate, total dose, time of pretreatment, and catabolic rate of the compound or compounds used in pretreatment will be continued. The basic evaluation will be made using AET and 5-HT in aqueous solution and then will be extended to these two drugs encapsulated in Dow-Corning silastic silicones. It is expected that this latter phase of the investigations will result in a more prolonged action of these drugs, thus enhancing their "protective action".

When completed, this phase of the investigations will permit initial evaluation of proposed "protective" agents on the basis of (1) their catabolic rates (2) their toxicity, and (3) their rate of release in experimental animals. More data will be obtained on mice irradiated at the center of the Co-60 source where the rate is approximately 100,000 r/min. This rate is the closest approximation to an "instantaneous" exposure that can be obtained with the Laboratory radiation facility. Preliminary experiments have already shown that the reactions of mice exposed to this high intensity radiation differ considerably from those observed at rates of only 80 r/min. These investigations will fill in the gap in the rate parameter between 1600 r/min and 100,000 r/min.

The computer program will be adapted to the new UCLA computer facility so that all calculation relative to source calibrations, to determination of mean survival times of experimental animals and statistical evaluation of so-called "protective" action of various procedures may be handled by the computer at a tremendous saving in time. In addition it is hoped that the basic program can be expanded as more data become available.

16. EXPECTED RESULTS IN FY 1966

With no major change in the scope of the program anticipated during FY 1966 it is expected that a quantitative evaluation of the factors that may modify the radiation syndrome will be possible. Furthermore it is hoped that it will be possible to present data on the late effects that may be found in experimental animals as the result of the protective action of AET and 5-HT.

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 - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
Isaac Harary

8. Project Term:
From: 1960 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	5	4 ½	4 ½
(b) Other Tech.	½	½	½
Total	5 ½	5	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 46,000	$ 43,900	$ 46,100
(b) Materials & Services	8,400	8,800	10,800
(c) Indirect Expenses* (4%)	40,600 (3%)	33,300 (3%)	34,000
Total	$ 95,000	$ 86,000	$ 90,900

* 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

Fujimoto, A., and Harary, I. In Vitro Studies on Single Beating Rat Heart Cell, IV. The Shift from Fat to Carbohydrate Metabolism in Culture. Biochem. Biophys. Acta, Vol. 82, 1964.

Kuramitsu, H., and Harary, I. In Vitro Studies on Single Beating Heart Cells. III. Enzyme Changes and Loss of Specific Function in Culture. Biochem. Biophys. Acta, Vol. 82, 1964.

Harary, I., and Sato, E. Studies In Vitro on Single Beating Heart Cells V. Changes in Adenosine-triphosphate-induced Contractions of Extracted Models. Biochem. Biophys. Acta, Vol. 82, 1964.

Harary, I., Fujimoto, A., McCarl, R., and Farley, B. Role of Lipids in Heart Cell Function. Federation Proceedings, Vol. 23, 1964.

Lewis, H., and Harary, I. Calcium-Activated Adenosine-Triphosphatase Localization in Cultured Beating Heart Cells. Science, July 1963, Vol. 141, pages 47-48.

Harary, I., Fujimoto, A., and Kuramitsu, H. Enzyme Changes in Cultured Heart Cells. Journal of the National Cancer Institute, 1964.

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. We are measuring the level of certain key enzymes such as those of glycolysis, the tricarboxylic acid cycle, fatty acid oxidation etc. to relate the activity of these enzymes to the specific function of the cell. As markers of specific function we use the ability to beat, the synthesis of muscle proteins such as myosin, and the visible subcellular structures, such as mitochondria and myofibrils. This is a project designed to investigate the control of metabolism tailored to bring about specific function and the synthesis of specific proteins. To this end we seek to determine the overall metabolic economy with regards to carbohydrates or lipids as fuels and to determine how this controls the rates and types of synthetic reactions in the cell.

Information of this sort will help us understand how the basic information of the gene is utilized to determine the selection of nucleic acids and proteins for the specific mammalian cell. Thus radiation effect on mammalian cells will be more adequately pinpointed and explained.

13. RELATIONSHIP TO OTHER PROJECTS

The following are workers in other laboratories investigating similar problems.
Wollenberger and Halle - Action of Cardiac glycosides on contractile activities in single cardiac cells.

Rhodin - Correlation of Ultrastructure and Organization.
Waddington and Perry - Polysomes in developing muscle cells.
Evans et al - Relation of Lipids to Heart Function.

14. TECHNICAL PROGRESS IN FY 1964

The purpose of our study of single heart cells in culture is to determine the relation of specific metabolism to specific function. We are studying enzyme levels, nutritional requirements and cell structure for pertinent information in this regard.

We have initiated a study of the serum requirements for both the growth and functioning of these cells. By extracting the serum with chloroform and methanol two serum fractions may be obtained, a lipid-free protein fraction and lipid extract. Cells grown in complete media grew and beat normally. They were maintained without growth or beating in a medium without serum. If however, the lipid extract was added to the medium without serum, the cells beat but did not grow. In another experiment cells incubated in a media with lipid-free serum grew partially but they did not beat. If the lipid extract was added the cells grew and beat as well as the control.

The most significant observation, relating substrate specificity to beating, is that lipid extract reinitiated beating when added to non-beating cells incubated in a lipid free medium.

The observations reported here link serum lipids directly with the beating of the heart cell. The metabolism of glucose in the presence of all the amino acids and vitamins normally found in the culture medium and the water soluble components of serum is not sufficient to support beating. Lipids may serve as a specific energy source and in this capacity may be involved in the determination of the specific function of the heart cell.

We have also developed a specific stain for calcium activated adenosine triphosphatase which is associated with contractile activity and a fluorescent antibody to myosin which specifically localized myosin in the single cell.

We have electronmicroscopic studies of the heart cells which show that these cells are synthesizing myofibrils perhaps through the action of the helically arranged polysomes which parallel the myofilaments.

15. EXPECTED RESULTS IN FY 1965

We will continue to study the relation of lipid metabolism to heart function. We expect to study lipid metabolism in the whole cell and in the mitochondria by following the ability to oxidize fatty acids and relating this ability to the functioning of the cell as measured by beating and level of myosin synthesis. We also intend to determine what lipid factors of the serum are required for function and to determine whether the lipid is utilized as a metabolite or as a synthetic intermediate. We will do this by following the pathway of added radioactive lipids to the cell medium

16. EXPECTED RESULTS IN FY 1966

We want to look more closely at the synthesis of actin and myosin in these systems. We are planning to isolate ribosomes from heart cells and to study the biosynthesis of myosin. We want to know more about the mechanism of synthesis of heart proteins so that we can see how lipid metabolism may control their

synthesis. It is our feeling that lipid metabolism may also control the expression of the genetic material necessary 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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	3	2	4
(b) Other Tech.	½	½	½
Total	3 ½	2 ½	4 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 26,100	$ 25,600	$ 47,400
(b) Materials & Services	6,100	4,800	8,100
(c) Indirect Expenses*	(2%) 20,300	(2%) 22,200	(3%) 34,000
Total	$ 52,500	$ 52,600	$ 89,500

* 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 1964

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, ultra-violet 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 1964

The serum protein-basic polypeptide interaction studies have been extended so that the stoichiometry of the poly-lysine serum albumin interaction has been elucidated and established under a variety of solvent conditions and solute concentrations as well as other environmental parameters. An hypothesis on the pathogenesis of certain aspects of acute and chronic disease has developed as a consequence of these discoveries and will be reported. In order to pursue the possibility of albumin binding being a reality, our laboratory has been set up to run immunoelectrophoretic analyses of various sera in agar gel. This will permit us, by the use of pure anti-human serum albumin as antibody to look for aberrant albumin in the Alpha-2 or Beta regions of the patterns. In the event that this is found this will be prima-facie evidence that such a mechanism is indeed operative.

The problem of instrumentation for the conformational analysis of proteins by optical rotatory dispersion in the ultra violet region of the spectrum has been resolved by the development of a superior spectropolarimeter distributed by the Bendix corporation. We are in the process of obtaining such an instrument, and hope that, if successful, we will be in a position to pursue more vigorously the conformational analysis of proteins in order to relate structure to activity in these important molecules.

15. EXPECTED RESULTS IN FY 1965

In the event that electrophoretically aberrant serum albumin can be detected by immunoelectrophoretic techniques an extensive clinical program will be begun in order to explore the nature of this component as a parameter of a variety of acute and chronic diseases. This will permit us to relate this, quantitatively, to disease states in order to derive an hypothesis on the nature of its origin. A micro-electrophoretic technique has been developed which will permit the electrophoretic analysis of the serum proteins in mice, by obtaining blood from nicked tail veins. Thus the serum proteins in individual mice can be studied as a parameter of developing Leukemia. Following the development of massive lymphoma the mice will be irradiated sufficiently to cause destruction of this radio-sensitive tissue and the effect of such massive tissue dissolution on the serum protein distributions will be measured. It is hoped that some evidence of albumin interactions will permit us to explore the possibility of nuclear histones being involved.

16. EXPECTED RESULTS IN FY 1966

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.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	4	4	4
(b) Other Tech.	1	1	1
Total	5	5	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 40,700	$ 47,100	$ 47,300
(b) Materials & Services	4,900	5,700	6,700
(c) Indirect Expenses*	(3%) 30,500	(3%) 33,300	(3%) 34,000
Total	$ 76,100	$ 86,100	$ 88,000

* 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 1964

Mead, J.F., and G.M. Levis. A-1 Carbon Degradation of the Long Chain Fatty Acids of Brain Sphingolipids. J. Biol. Chem. 238, 1634 (1963)

Mead, J.F., and G.M. Levis. Enzymatic Decarboxylation of the Alpha-Hydroxy Acids by Brain Microsomes. Biochem. Biophys. Res. Comm. 11, 319, (1963).

Kayama, M., Y. Tsuchiya, and J.F. Mead. A Model Experiment of Aquatic Food Chain with Special Significance in Fatty Acid Conversion. Bull. Japan Soc. Scientific Fisheries 29,452 (1963).

Malamos, B., C. Miras, and J.F. Mead. Effect of Ionizing Radiation on Lipid Metabolism of Blood Cells In Vitro. Nature, 198, 401 (1963).

Kayama, M., Y. Tsuchiya, J.C. Nevenzel, A.J. Fulco, and J.F. Mead. Incorporation of Linolenic-1-C^{14} Acid into Eicosapentaenoic and Docosahexaenoic Acids in Fish. J. Am. Oil Chem. Soc. 40,499 (1963)

J.F. Mead. Lipid Metabolism, pp. 341-268 of Annual Reviews of Biochemistry Vol. 32 (1963) E.E. Snell, Editor.

E.C. Horning, E.H. Ahrens, Jr., J.R. Lipsky, F.H. Mattson, J.F. Mead, D.A. Turner and W.H. Goldwater. Quantitative Analysis of Fatty Acids by Gas-Liquid Chromatography. J. Lipid Res. 5,20-28 (1964).

J.S. O'Brien, D.L. Fillerup and J.F. Mead, Brain Lipids: I. Quantification and Fatty Acid Composition of Cerebroside Sulfate in Human Cerebral Grey and White Matter. J. Lipid Res. 5,109-17 (1964).

C.S. Davidson, E.H. Ahrens, Jr., D.S. Fredrickson, R.T. Holman, C.S. Lieber, J.F. Mead, B.N. Miller, and T.B. van Itallie. The Role of Dietary Fat in Human Health, A report of the Food and Nutrition Board, National Academy of Sciences, National Research Council.

G. Levis and J.F. Mead. An Alpha-Hydroxy Acid Decarboxylase in Brain Microsomes. J. Biol. Chem., 239, 77 (1964).

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 especial 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

See listing of General Metabolism and Chemical Radiobiology Sections; studies at the Hormel Institute, University of Minnesota, Austin, (W.O. Lundberg); Los Alamos Scientific Laboratory (H. W. Langham); "Behavior of Cell Membrane Mechanisms During and Following Gamma Radiation", G.H. Bourne, Emory University.

14. TECHNICAL PROGRESS IN FY 1964

By the use of the chromatographic techniques continuously improving during the year, several aging human brains have been completely analyzed. The following lipids have been analyzed, including fatty acids, where applicable: cholesterol, cerebroside, ceramide, cerebroside sulfate, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, several inosital phosphalipids, ganglioside and sphingomyelin. Several unidentified lipids are being characterized and may be of importance in aging. Although it is still too early to recognize trends occurring with age with any certainty, this information should be available soon. It is to be expected that in nervous tissue, especially the white matter of the central nervous system in which turnover is very slow, changes with aging should be easily recognizable.

If this supposition proves to be the case, a much better criterion of aging than longevity will be established. Factors that in certain respects mimic aging, such as chronic irradiation, will then be assessed in their true light.

Meanwhile, certain interesting relationships between the brain lipids and their components have been established and the function of these lipids in brain activity can be considered.

The α-hydroxy acid decarboxylase, which appears to act as a scavenger for the very long-chain fatty acids possibly released during aging and demyelinating disease, has been located in the brain microsomes and partially characterized. The requirements of the system have been investigated and an unknown cofactor has been located in the 100,000xg supernate.

15. EXPECTED RESULTS IN FY 1965

During this year it is expected that the analysis of control brains will be completed and that those lipids that have been shown to be important in aging will have been characterized. Moreover, the effect of irradiation on the human brain and of chronic irradiation on the rat brain will considered.

The α-hydroxy acid decarboxylase will be further characterized and the mechanisms of this interesting reaction clarified. Moreover, the initial reaction, hydroxylation of the very long-chain fatty acids in the brain will be investigated. These studies should reveal the importance of this reaction in the again process of the brain. Further studies on the formation and deposition of the polyunsaturated fatty acids in animal tissues will be considered with emphasis on their function in membranes.

16. EXPECTED RESULTS IN FY 1966

It is anticipated that future research will continue along lines begun during the current period and depending on results obtained during this time. Thus, the measurement of the effects of aging and irradiation on the brain lipids should have given definite information and will have pointed the way to continued study. Tracer studies of the enzymatic reactions of lipid metabolism should continue with emphasis on the reaction of hydroxylation and desaturation. The study of the "aging pigment" and its relationship to chronic irradiation will be continued.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	2	2	2
(b) Other Tech.	½	½	½
Total	2 ½	2 ½	2 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 26,700	$ 28,600	$ 28,600
(b) Materials & Services	2,700	3,800	3,800
(c) Indirect Expenses* (2%)	20,300	(2%) 22,200	(2%) 22,700
Total	$ 49,700	$ 54,600	$ 55,100

* 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 1964

"Nature of the Products Formed by γ-Irradiation of Deaerated Aqueous Potassium Oleate", David R. Howton, Radiation Research, 20,161 (1963).

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 primary interest. Inasmuch as the usually unbranched saturated or cis-unsaturated hydrocarbon chains they contain are 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.

In-sight 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 otherwide 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 un-equavocal degradative techniques. Fundamental studies of adsorption and light absorption phenomena 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.; and "Effect of X-Ray Radiation on Lipids of Skin", H.P. Schwarz, Phila. Gen. Hospital.

14. TECHNICAL PROGRESS IN FY 1964

Major experimental effort has been directed toward improving understanding of the direct effects of ionizing radiation on the hydrocarbon moieties of lipids, using free fatty acids (oleic and stearic) as model substances. It has been recognized for some time that such agents bring about ostensibly straight forward decarboxylation (in addition to other reactions), giving carbon dioxide and hydrocarbon. In 1952, Burton and Breger (Science, 116, 477) reported that deuteron irradiation of oleic acid yields trans-8-heptadecane - a surprising

result with respect to the implication that isomerization of the originally cis double bond accompanies decarboxylation. Comparison of the infrared absorption spectrum published by Burton and Breger with those of authentic samples of both the cis and trans isomers of this hydrocarbon suggests strongly that the product is in fact principally the cis isomer, together with small amounts of the trans isomer. (presence of some terminal unsaturation is also indicated). γ-Irradiation of oleic acid yields hydrocarbon displaying infrared absorption very similar to that reported for the product of deuteron irradiation. Gas chromatographic studies revealed that hydrocarbons of considerably greater chain length (probably C_{34}) are also produced, and that the "unchanged methyl oleate" fraction obtained by silicic acid chromatography of the total (diazomethane-treated) products contains some stearate, together with some material containing trans unsaturation. Most of the altered oleate, however, exhibits increased adsorption affinity for silicic acid, suggestive of polymeric nature. Experiments involving doses of 0.5-7.0 X 10^8r disclose G_{oleate} 9.6 (in accord with suspected extensive polymerization by chain reactions; all yields are those obtained by extrapolation to zero dose), $G_{hydrocarbon}$ 0.67, and $G_{stearate}$ 0.42; the yield of trans unsaturation in recovered monostearic ester closely parallels that of stearate. On the basis of known consequences of hydrocarbon irradiation, it was suspected that this trans olefinic material might owe its infrared absorbing properties to new unsaturation rather than to isomerized cis double bonds, in which case products of stearic acid irradiation should also contain trans unsaturation. Both hydrocarbon and "recovered stearate" from stearic acid samples irradiated under identical conditions (i.e., identical to those employed with oleic) were found to be quite free of unsaturation (implying that oleic is indeed isomerized to elaidic and to some extent). Quite unexpectedly, however, the yield of hydrocarbon from stearic acid was found to be about seven times that from oleic (at 4.5 X 10^8r). These observations have been interpreted as indicating that both double bond and carboxyl groups function very effectively as "electron-hole" traps, in essence "protecting" the saturated hydrocarbon portions of such molecules from chemical changes which would otherwise be typical under such conditions; absence of trans unsaturation in the hydrocarbon product from stearic acid irradiation indicated that intermolecular electron-hole migration is also an efficient process. Obvious implications of these observations are being explored.

15. EXPECTED RESULTS IN 1965

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 extent of intermolecular electron-deficiency transfer by analysis of hydrocarbons obtained by irradiation of stearic acid in the presence of different amounts of n-hexadecane; 2) determination of the relative effectiveness of double-bond and carboxyl groups as electron-deficiency traps in terms of hydrocarbon yield versus position of double bond in the unsaturated fatty acid molecule; 3) definition of the mechanism of the decarboxylation reaction by establishing the nature of concomitantly formed products and examination of suitable model systems.

16. EXPECTED RESULTS IN FY 1966

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 next to more complex esters, 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 - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
J. D. Nevenzel

8. Project Term:
From: 1962 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2	2	2
(b) Other Tech.	½	½	½
Total	2 ½	2 ½	2 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 22,900	$ 24,200	$ 25,300
(b) Materials & Services	2,700	2,900	2,900
(c) ~~Indirect~~ Expenses* (2%)	20,300 (2%)	22,200 (2%)	22,700
Total	$ 45,900	$ 49,300	$ 50,900

* 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 1963

"Incorporation of Linolenic-1-C^{14} Acid into Eicosapentaenoic And Docosahexaenoic Acids in Fish", M. Kayama, Y. Tsuchiya, J.D. Nevenzel, and A. Fulco, and J.F. Mead, J.Am.Oil Chemists' Society, 40,499 (1963).

"The Fatty Acid Composition of Three Marine Invertebrates", W. Rodegker and J. D. Nevenzel, Comp. Biochem. Physiol. 11,53 (1964).

12. SCOPE OF THE PROJECT

The Tracer Synthesis Section was established to prepare isotopically labelled molecules of biological or chemical interest when required for use in the 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 lipids in higher plants, with particular emphasis on the biogenesis of the long-chain constituents of plant cuticle waxes. By using adsorption column, thin layer, and gas liquid chromatographic methods for the separation and analysis of such consituents, and carbon-14 or tritium labelling to trace the pathways involved in their biosynthesis comprehensive investigations can be carried out with the wax obtainable from only a few plants. The results of the leaf wax investigation: (1) will provide insight into the basic biochemistry of several new types of lipids in higher plants; (2) may be significant for the study of brain lipids during aging, now under way in the General Metabolism Section of this Division, since in both fields compounds of chain lengths longer than the usual C_{16} to C_{20} are involved, and one-carbon rather than the usual two-carbon intermediates seem to be important; and (3) will 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.

In support of these programs the Tracer Synthesis Section is developing new methods for the analysis, separation, and controlled degradation of lipid constituents, and in applying these methods to new areas of research. An example is a new gas chromatographic method for the analysis of the α-alkyl glyceryl ethers (which are now being investigated as potential radiation protection 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 biochemical studies of this class of lipids, their distribution and metabolism. 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 monoglycerides and the vicinal diols, 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 1964

The characterization of a major fraction of the cutical wax of the ornamental rubber plant, Ficus elastica, as long-chain aldehyde has been firmly established by (1) oxidation to the corresponding normal fatty acids; (2) reduction to both the alcohols and, further, to the normal alkanes, and GLC analysis of each of these three derived products; and (3) formation of a derivative, the 2-alkyl 1,3-diphenyl-imidazolidine, by a reaction specific for aldehydes. By the use of rooted individual leaves it has been possible to observe the incorporation of carbon-14 from radioactive acetate into the leaf wax of F. elastica, although it was necessary to trim the roots to 1-2 mm. in length in order to get passage of activity through the roots. Even so only about 0.1% of the total activity metabolized was found in the wax and about 3.2% in the remaining lipid from the leaf. (In a preliminary experiment in which the roots were 2-4 cm. in length, no activity was detected in the leaf wax, but the root lipid has a specific activity of 1.7×10^3 d/s/mg). Improvements in experimental conditions, and a 10-fold increase in the total activity used are expected to produce sufficiently high specific activities in the wax components to permit degradative studies to establish labelling patterns. Two additional plants surveyed are promising for future research, Hebe andersonii, an ornamental shrub common in Southern California, and Larrea divaricata, the creosote bush, from the Nevada Test Site.

The work on the lipids of marine animals was extended to include the mussel, Mytilus californianus, a sponge (probably Verongia thiona), and the Gemphylidae fish, Lepidocybium flavobruneum. The lipids of the latter are unusual in their abundance in the muscle (19-20% of the wet weight, or over 50% on a dry weight basis) and in the predominance of wax esters in the lipids from red and white muscle (88% of the total lipid) and the spleen. A paper on the fatty acid composition of the marine invertebrates Pisaster ochraceus, Mitella polymerus, and Mytilus californianus was published early in 1964.

The gas chromatographic method for the analysis of glyceryl ethers has been submitted for publication, including an analysis of the glyceryl ethers of dogfish liver oil (Squalus acanthias).

15. EXPECTED RESULTS IN FY 1965

In FY 1965 it is expected that the investigation of the biosynthesis of leaf wax constituents in F. elastica will reach the stage where labelling patterns for the hydrocarbons and aldehydes from administered acetate-C^{14} will be established.

The use of a yeast, Torulopsis magnoliae, to degrade the long-chain wax constituents to C_{17} and C_{18} fatty acids will be investigated.

If a suitable small fish can be obtained, the metabolism of the wax esters will be studies in vivo (work to be done with the General Metabolism Section). The white muscle in fish is poorly supplied with blood, and it is not clear how aerobic reactions can provide sufficient energy for sustained activity. In Lepidocybium the additional question arises whether the concomitant high content of wax esters is directly related to the source of energy utilized by the muscle. We hope to elucidate these basic points. The plant leaf wax research and the work on the lipids of marine organisms will converge in this area, allowing a comparison to be made of the biosyntheses of long-chain alcohols in leaf waxes and in the fish wax esters (see Item 16).

16. EXPECTED RESULTS IN FY 1966

In FY 1966 the origin of the alcohols of leaf waxes of F. elastica and wheat will be under investigation. For this purpose wheat offers the advantages that the alcohols constitute 47% of the total wax and that radiation-produced mutants with altered leaf 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 arranced, 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.

Also, work on the metabolism of the wax esters in fish are expected to have reached the point where a comparison between the biosynthesis of the alcohol moieties of the fish wax esters and of the alcohols of leaf waxes will be possible.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	3 ½	3	3
(b) Other Tech.	½	½	½
Total	4	3 ½	3 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 25,300	$ 26,400	$ 27,500
(b) Materials & Services	5,500	3,500	3,500
(c) Indirect Expenses* (2%)	20,300	(2%) 22,200	(2%) 22,700
Total	$ 51,100	$ 52,100	$ 53,700

* 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 FISCAL YEAR 1964

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 1964

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

15. EXPECTED RESULTS IN FY 1965

The Pathology services will continue essentially unchanged.

16. EXPECTED RESULTS IN FY 1966

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	6 ½	6 ½	6 ½
(b) Other Tech.	2 ½	2 ½	2 ½
Total	9	9	9

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 68,700	$ 79,200	$ 82,500
(b) Materials & Services	17,000	10,600	11,600
(c) Indirect Expenses*	(6%) 61,000	(5%) 55,500	(5%) 56,700
Total	$ 146,700	$ 145,300	$ 150,800

* 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 1964

(a) Radiation Chemistry of Thymine in Aqueous Alkaline Solution. L.S. Myers, Jr., W.T. Tsukamoto, J.F. Ward, J.R. Julca, and D.E. Holmes, Absts. of Papers, 145th A.C.S. Meeting, September 8-13, 1963, p. 9 T.

(b) Effect of Inorganic Anions on some Radiation Chemical Yields in Aqueous Solution. J.F. Ward and L.S. Myers, Jr., Absts. of Papers, 145th A.C.S. Meeting, September 8-13, 1963, p. 14 T.

(c) Structural Effects in the Radiation Induced Reactions of Phyrimidine Compounds. L.S. Myers, Jr., W.T. Tsukamoto, J.F. Ward, and D.E. Holmes. Absts. 8th Ann. Meeting of the Biophysical Society, Feb. 26-8, 1964. p. W. D. 12.

(d) The Radiation Chemistry of Papain in Dilute Aqueous Solutions, Radiation Research 21. (1964) (UCLA-517).

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.R. 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 1964

Effects of Ionizing Radiation on Thymine

Because of its pertinence to studies of radiation damage of DNA an extensive investigation of the effects of radiation on thymine, the pyrimidine base peculiar to DNA, is in progress. Previous work has shown that in slightly acid or neutral aqueous solutions the principle site of radiolytic attack on thymine is the 5,6 double bond, with ultimate formation of saturated ring compounds. As the pH is increased through and beyond the physiological pH region the yeild of this reaction decreases and the site of attack shifts to the 5-methyl group. In strongly alkaline solutions the attack is almost exclusively on this group. Further investigations of this reaction have involved development of new analytical techniques which are reliable in alkaline solutions, and use of these techniques to study reaction products. Previous preliminary evidence that the principle product of attack on the methyl group is hydroxymethyl uracil has been confirmed and evidence has been obtained which indicates that the hydroxymethyl uracil is an immediate, not a secondary, product of the reaction. In addition, several other as yet unidentified products have been found in small yield, and preliminary attempts have been made to develop a reaction mechanism.

The Relative Sensitivity of Pyrimidine Compounds to Radiation

The discovery that the nature of the indirect action of radiation on thymine changes as the pH is increased through and beyond the physiological region makes it almost certain that other phyrimdine compounds will show similar changes, and that their relative sensitivity as determined by any single criterion will depend critically on the experimental conditions. A set of compounds was therefore selected (uracil, thymine, cytosine, and 5- methylcytosine, their nucleosides, and their nucleotides) and their sensitivity to the indirect action of cobalt -60 γ-rays was investigated spectrophotometrically in neutral solutions and at the pH extremes to determine the maximum extent of variation in sensitivity and the relation of this variation to amino, methyl, pentose, and pentose-phosphate substituents. In acid and neutral solutions, in which the pyrimidines are positively charged or neutral, the initial disappearance yield of the chromophore (or the 5,6 double bond) was found to be about the same for thymine, uracil, cytosine, 5-methyl cytosine, and the corresponding nucleosides, approximately 3 molecules/100 e.v. absorbed. It is also the same for cytidylic acid in acid, but somewhat lower in neutral solution. In alkaline solution, in which the pyrimidines are negatively charged, the results are complex and depend markedly on the side chains. Thus with uracil and cytosine the yields are initially about the same as in acid, but rapidly decrease with increasing dose. They are essentially zero with compounds which have a methyl group in the 5 position. In compounds which have a sugar attached to the ring, the disappearance yield is again zero if a 5-methyl group is present. If it is not, the intensity of the chromophore increases when the nucleosides are irradiated. A similar increase was obtained with cytidylic acid. These results are consistent with the view that in the positively charged or neutral molecules the 5,6 double bond is the most sensitive group to irradiation but that in the ionized forms the methyl and sugar groups are also attacked if present. The relative order of reactivity appears to be methyl and

ribose > 5,6 Double Bond > Deoxyribose.

Preliminary Investigations of Radiation Effects on Biologically Important Compounds

Considerable evidence suggests that direct as well as indirect effects of ionizing radiation play an important part in radiation damage to nucleic acids. Preliminary experiments have therefore been done in which solid Carbon-14 labeled pyrimidines and nucleosides have been exposed to cobalt-60 γ-rays and analyzed by paper chromatography. It is apparent that a number of products result. Further experiments are needed to identify these products and to determine the conditions affecting their formation.

Preliminary experiments are also under way with model compounds to determine the possible role of "bound" water in radiation effects.

Development of the Radiation Facility

Continued progress has been made in developing the new 10,000 curie cobalt-60 source as a versatile tool for use in the Radiobiological Sciences. Several devices required to meet specialized experimental needs have been designed and built, and equipment for routine exposures under highly reproducible conditions has been installed. In addition operation of 250 kvp and 50 kvp X-ray machines has been continued for experiments requiring less penetrating radiation than that of cobalt-60. Also, a 150 curie cobalt-60 source, which formerly was our only source of highly penetrating radiation, is still used for test tube experiments which do not require a high intensity radiation field.

Experiments with Nucleoproteins and Protein Constituents

This work is reported by Dr. J.F. Ward under the Project Title "Chemical Radiobiology II".

15. EXPECTED RESULTS IN FY 1965

Investigations of the mechanisms of the radiolysis of biologically important compounds will continue with emphasis on the components of nucleoproteins. Further investigations of radiation effects on thymine will be undertaken to obtain additional information relative to identification of products and the influence of oxygen on the formation of products, but decreasing emphasis on this compound is anticipated. As a logical development from the work on thymine, increasing emphasis will be placed on studies of other pyrimidines, the purines, nucleosides, nucleotides, nucleic acids, and possibly nucleoproteins. The highly successful preliminary experiments on the direct effects of radiation on nucleic acid constituents will be continued and extended to other compounds and considerable progress is anticipated towards elucidation of the reaction mechanisms involved. Further studies will also be pursued on the importance of "bound" water on radiation effects in model compounds, and, if progress permits, will be extended to biologically important molecules themselves. Efforts to develop further the new 10,000 curie cobalt-60 source as a versatile tool for radiobiological research will be continued.

16. EXPECTED RESULTS IN FY 1966

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 - 1964

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: 1947 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2	2	2
(b) Other Tech.			
Total	2	2	2

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 16,300	$ 18,700	$ 19,800
(b) Materials & Services	6,000	4,000	4,000
(c) Indirect Expenses*	(1%) 10,200	(1%) 11,100	(1%) 11,300
Total	$ 32,500	$ 33,800	$ 35,100

* 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 1964

(1) Radiation Chemistry of Thymine in Aqueous Alkaline Solution. L.S. Myers,Jr. W.T. Tsukamoto, J.F. Ward, J.R. Julca, and D.E. Holmes. Absts. of Papers, 145th A.C.S. Meeting, September 8-13, 1963, p. 9 T.

(2) Effect of Inorganic Anions on some Radiation Chemical Yields in Aqueous Solution. J.F. Ward and L.S. Myers, Jr., Absts. of Papers, 145th A.C.S. Meeting, September 8-13, 1963, p. 14 T.

(3) Structural Effects in the Radiation Induced Reactions of Pyrimidine Compounds. L.S. Myers, Jr., W.T. Tsukamoto, J.F. Ward, and D.E. Holmes. Absts. 8th Ann. Meeting of the Biophysical Society, Feb. 26-8, 1964, p. W.D. 12.

12. SCOPE OF THE PROJECT

This project involves investigation of the site within the cell of the primary significant effect of ionizing radiation and requires a knowledge of the sensitivities and mechanisms of radiation destruction of biologically important macromolecules such as nucleoproteins, transforming principle DNA and enzymes. Correlation of the decrease in ability of these macromolecules to function biologically with chemical changes produced by irradiation is necessary in order that the biological effects can be understood on a quantitative radiation chemical basis. Studies of simple model compounds are often required to make such correlations possible.

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 J. Leitch in this Laboratory. Studies in a similar vein are being carried out throught 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; 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, Australia, Sweden and Japan.

14. TECHNICAL PROGRESS IN FY 1964

Effect of Ions on Radiation Yields in Simple Biochemical Systems

Living cells contain appreciable concentrations of ions, irradiation of the cell produces free radicals from the water which may react with these ions. It is known for instance that under certain conditions chloride ions will react with OH free radicals:

$$OH^{\cdot} + Cl^{-} \longrightarrow Cl^{\cdot} + OH \qquad (1)$$

The chlorine atoms thus produced are less reactive than OH free radicals and hence more specific in their mode of reaction, i.e., chlorine atoms will add to double bonds but will not abstract hydrogen atoms, whereas OH free radicals will carry out both of these reactions.

In a system which contains both a double bond and a saturated compound, OH free radicals will react with both components, if chloride ions present in the system react with the OH free radicals, a specificity for attack on the double bond component will be observed. Using such a system and varying pH and sodium chloride concentrations it was seen that the above reaction (1) occurs only below pH 3.0. The kinetics of the system seem to fit a mechanism:

$$OH^{\cdot} + Cl^{-} + H^{+} \longrightarrow H_2O + Cl^{\cdot} \qquad (2)$$

Relative rate constants of the reaction of the OH free radical with the components of the system were calculated and using these it was possible to fit theoretical data closely to the experimental results.

Hence this reaction of chloride ions is not important at physiological pHs, this was borne out when solutions of DNA and transforming DNA were used. There was very little effect of chloride (as compared to sulphate) on the radiation induced destruction of DNA bases, hyperchromicity and transforming activity.

In the case of bromide ions, however, bromine atoms are produced by reaction with OH free radicals in neutral as well as acid solution. These bromine atoms are much less reactive than the OH free radicals and will not add to double bonds. Consequently bromide ions protect compounds from the effect of OH free radicals. In the case of DNA - assayed chemically for base destruction and hyperchromicity and biologically for transforming activity a marked protection occurs - in the case of transforming DNA a 100 fold decrease in the effect of the radiation occurs.

Compounds Containing Sulfhydryl Groups

Sulfhydryl compounds are known to be sensitive to ionizing radiation in aqueous solution and are used as protective agents. We have established that this sensitivity is due (for cysteine) to reaction of the sulfhydryl group with O_2^- ion radicals followed by a short chain reaction in which more sulfhydryl groups are destroyed. Work has commenced on papain to see if this situation exists when the sulfhydryl group is present in a macromolecule. It appears however, that the majority of the deactivation of papain caused by ionizing radiation is produced by reaction with the OH free radical. This has been shown by simple chemical competion experiments and by the use of bromide ion (vide supra) which does protect papain.

15. EXPECTED RESULTS IN 1965

Investigations will continue in trying to find the important sites of the primary attack of ionizing radiation in the cell. The relative sensitivities of compounds irradiated in quasi-physiological conditions will be one line of approach, another ill be attempts to discover the importance of various chemical changes in producing damage visible on a cellular level.

For instance work with transforming principle DNA in solution will be pursued through to irradiation of nucleoprotein, cell nuclei and cell suspensions, using

the activity of the DNA as a measure of the biological damage observed.

Model compounds such as polyadenylic acid will be required, since at the moment the stoichiometry of destruction of purine moieties within the DNA in aqueous solution by ionizing radiation is poorly understood.

Analogous experiments will be carried out in proteins, nucleoproteins and enzymes. Emphasis will be placed on proteins (enzymes) having a well defined prosthetic group, i.e., papain. Hence these radiation-biochemical studies of indirect damage to enzyme molecules will lead to an assessment of possible protecting chemicals and to the estimation of the importance of direct damage by radiation.

16. EXPECTED RESULTS IN 1966

The use of biological assays of cellular components extracted after irradiation in vivo and investigation of the chemical damage responsible will lead to an understanding of ways in which cell damage can be modified. Much of the work will be involved in constructing model systems which simulate physiological conditions, and devising justifiable extrapolations to in vivo systems.

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 - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
Ole A. Schjeide

8. Project Term:
From: 1952 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	4	5	5
(b) Other Tech.	½	½	½
Total	4 ½	5 ½	5 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 37,400	$ 53,900	$ 56,100
(b) Materials & Services	11,000	9,000	11,400
(c) Indirect Expenses* (3%)	30,500	(4%) 44,400	(3%) 34,000
Total	$ 78,900	$ 107,300	$ 101,500

* 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 1964

(a) Effects of Various Factors on Occurrence of Intra-Mitochondrial Inclusions. O.A. Schjeide, Ruth G. McCandless, Myrna Wilkens, Marion Peterson and G.V. Alexander. Exp. Cell Research 32, 379-390 (1963).

(b) Further Observations on the Developing Avian Oocyte. Origins and Roles of Mitochondria-Like Organelles. O.A. Schjeide, Ruth G. McCandless and R.J.Munn. Growth 27, 111-124 (1963)

(c) Possible Participation of RNA in Formation of Mitochondria-Like Organelles. O.A. Schjeide, Ruth G. McCandless and R.J.Munn. Growth 27, 125-128 (1963).

(d) Effects of Acid Mucopolysaccharides on Growth Rates and Constituent Lipids of Tissue Cultures. L.M. Morrison, O.A. Schjeide, J.J. Quilligan, Jr., L. Freeman and R. Holeman. Proc. Soc. Exp. Biol. Med. 113, 362-366 (1963)

(e) Parameters of OEstrogen-Stimulated Protein Synthesis. O.A. Schjeide and Myrna Wilkens. Nature 201, 42-44 (1964).

(f) Synthesis of Cytoplasmic Hematin by Nuclei of Erythrocytes from Embryos. O.A. Schjeide, G.V. Alexander, J.P. OKunewick, C.R. Carmack, M. Wilkens, E.N. Carlsen and T.G. Hennessy. Growth, March (1964).

(g) Relationships Between Hematin Synthesis and Morphological Structures in Adult and Fetal Red Cells. O.A. Schjeide, Ruth G. McCandless and R.J. Munn. Growth, March (1964).

(h) Phospholipid Synthesis in the Erythrocyte Nucleus. O.A. Schjeide, R. Marshall and Myrna Wilkens. Nature (April 4, 1964).

(i) Transfer Ribonucleic Acid. E.N. Carlsen, G.J. Trelle and O.A. Schjeide. Accepted by Nature (April, 1964).

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.

Specifically, the section concerns itself with such problems as the metabolism and syntheses of the nucleus in relation to other subcellular structures, the origins and modes of propagation of various subcellular structures, the nucleic

acid systems involved in the production of proteins, the control of protein and lipid syntheses by various hormones, the intracellular sites of hormone action and the passage of macromolecules across cell membranes. Whenever possible, the effects of radiations on such processes are assessed and, in most cases, the studies are carried out on tissues at different levels of differentiation.

Various theories are being developed with respect to the roles of oxidizing radicals in aging and disease.

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 1964

During 1964 the Section has accomplished the following:

Effects of Estrogens on Proteins and Lipids

(A) Two separate effects of estrogen on the liver of the chicken have been noted.

1. The liver is stimulated to produce two new plasma proteins, phosvitin and lipovitellin. These are first detectible in the serum 24 hours after estrogen injection; they are ejected from the liver as rapidly as they are formed; and their appearance is correlated with a rise in the total liver RNA.

2. The liver is induced to synthesize and eject additional quantities of triglyceride into the plasma, where it is present in the form of light lipoprotein. The increased triglyceride is detectible in the serum as early as 12 hours post-estrogen injection and is placed immediately into the serum.

Both the unique proteins and the light lipoprotein enter the egg yolk - the unique proteins into the yolk granules and the light lipoproteins into the fluid in which the granules are suspended. At some as yet undetermined point in passage, the granule proteins are reduced in molecular weight and are rendered insoluble.

The lipid moiety of the light lipoprotein also undergoes some changes in composition (Ref. Nature 201, 42-44 (1964).

Effects of Estrogens on Carrier RNAs

Estrogen treatment results in the production by the avian liver of large quantities of 2 plasma proteins containing extraordinary proportions of serine and phosphoserine. A system is thus available for the study of carrier - or transport - RNAs specific for these two amino acids (which have been largely neglected by workers in the field of mechanisms of protein synthesis).

By the employment of an albumin column, a partial resolution of transfer RNAs from estrogenized and control livers has been accomplished. Although the total quantities of carrier RNA per unit dry weight of liver are the same, proportionately higher concentrations of carrier RNAs specific for serine and phosphoserine have been detected (by labeling with C^{14} serine and P^{32} and observing the positions of the labels in the column fractions) in the livers of estrogenized chickens. Turnover of these RNAs is much more rapid in estrogenized chickens than is the turnover of carrier RNAs for other amino acids.
A basic principle that can be derived from these experiments is that the synthesis of a protein is not solely governed by the messenger RNA made available for such synthesis but that the carrier RNA system is also immediately subject to genetic regulation and may play a critical role in at least the rate of synthesis of a given protein.

The demonstration of carrier RNAs specific for phosphoserine indicates that phosphorylation of serine in the proteins under study takes place prior to the formation of the proteins rather than after (Ref. Nature, May or June, 1964).

Nuclear Synthesis

The nucleated avian erythrocyte is representative of a cell type in which nuclear events appear to comprise the bulk of the cell's total metabolism. Electronmicrographs reveal that very little ribosomal material, no golgi apparatus, no endothelial reticulum and only a few mitochondria are present in the cytoplasm. In red cells from younger embryos there is more RNA and DNA per nucleus; there are more mitochondria and many of these are inserted into the nuclear pores. In vitro incubation studies with Fe^{59} show that hematin is synthesized in the nucleus and this 30 times more rapidly in cells from 7 day embryos as compared in cells from chicks. Turnover of nuclear phospholipid (P^{32} used) is as much as 6 times faster in cells from 11 day embryos as compared to those from chicks. Preliminary studies with C^{14} lysine indicate that the globin moiety of hemoglobin is also synthesized in the nuclei of erythrocytes. Most of the hematin, synthesized in the nucleus, is quickly passed to the cytoplasm. Addition of serum or glycine to the incubation medium speeds synthesis of hematin but slows turnover of RNA and phospholipid. The fact that an inverse relationship exists between hematin synthesis and turnover of total RNA suggests that hemoglobin is being synthesized on long lasting messenger RNA or that hematin synthesis may proceed independently of globin synthesis.

The respiration rates of red cells from 11 day embryos appear to be only twice as rapid as in cells from chicks. It has not yet been determined whether respiration takes place solely in the mitochondria or whether the nuclei of these cells might also be involved. (Ref. Growth, March, 1964).

Effects of Radiations on Myelin Formation

The brain stems of rats that have received 750 r of X-irradiation to the head only (at two days after birth) show a decrease in total weight as compared to controls, a condition which is apparent at about 2 weeks and persists throughout the life span of the animal. Since approximately 80 per cent of the deficit in weight can be accounted for in terms of lipid alone, it was suspected that a profound radiation-induced inhibition of myelin formation has taken place in these young rats.

Histological examination of the 5th nerve tract by means of both electron microscopy and the light microscope reveals that there is an initial retardation of division in the glial cells of this tract. However, the cell division eventually not only equals the rate occurring in normal brain stem but exceeds it, so that at 60 days of age the fifth nerve tract of the irradiated animal contains as many total glial cells as that of the control, although the volume of the tract is considerably smaller (20-30 per cent).

Each glial cell in the fifth nerve tract of the irradiated 30-day-old rat is much smaller (both cytoplasm and nucleus). Many of them are wrapped about nerve fibers and the newly forming - or incompletely developed - myelin sheath is seen to be well within the boundaries of the cytoplasms of the glial cells, a condition that rarely obtains in the normal animal. Many of the nerve fibers have no myelin about them and those that do, have thinner layers. The myelin itself appears to be amorphous in many cases in the irradiated rats whereas in the control animal a precise resolution into membrane layers is seen around all nerve fibers.

Biochemically - the total brain stems of head-irradiated animals contain relatively more cholesterol and less total phospholipid after two weeks of age than do those of control litter mates. A normally occurring increase in oleic acid fails to take place and may reflect an effect on lipid dehydrogenase systems. Aldehydes, which indicate the presence of certain plasmologens of myelin fail to materialize. There appear to be some differences in proportions of constituent phospholipids but this data is tentative.

Nucleo-cytoplasmic Exchanges

Electron microscopy of developing avian oocytes has indicated that the so called "annular pores" of the nuclear membrane are not open holes although the roller-like rods making up the annuli may be so. A common mode of transfer of nuclear materials to the cytoplasm through them is by disassociation of vesicles arriving at the pore site on the nuclear side into very much smaller vesicles and the re-emergence of small vesicles on the cytoplasmic side which re-assemble into larger vesicles (resembling "kinetosomes" as seen in Paramecium). The "kinetosomes" appear to move freely from cell to cell, traversing the two cell membranes by a dissassembly-reassembly process similar to that described for passage across the nuclear membrane.

At least some of the vesicles traversing the nuclear membrane are observed to be present as such in the nucleolus where they are sometimes arranged in helical stacks 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.

Nuclear Regulation of Organelle Formation

The morphogenesis of mitochondria-like organelles, conventional mitochondria, yolk granules, golgi apparatus, endoplasmic reticulum and plasma membrane in cells of avian species have all been found to be intimately related to vesicles in the cytoplasm which initially bear strong resemblance to the (vesicles) "kinetosomes" arising from the nuclear-cytoplasmic interface.

In each case the formation of the new organelle appears to be accompanied by disassociation of the original kinetosome into sub-vesicles which further dis-assosiate into microvesicles. The microvesicles either fuse into membrane pairs (such as cristae) or elaborate (in an unknown manner) to produce membrane sheets.

A concept emerging from these studies is that information for the formation of a complex organelle in the cytoplasm is transmitted in a vesicular package from the nucleus and that expression of this information is dependent on substrate available and stimulators and repressers in the cytoplasm. The information in the large package would appear to be arranged in repeating modules so that expression is realized through sub-divisions within each module. It is noted that all of the organelles found to be related to vesicles consist of repeating units of structure and activity.

15. EXPECTED RESULTS IN FY 1965

During 1965, 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 irradiated brain stems of neo-natal rats.

16. EXPECTED RESULTS IN FY 1966

Continuation of 1965 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 - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
N. de T. Whitaker

8. Project Term:
From: 1961 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	1	3	4
(b) Other Tech.			
Total	1	3	4

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 10,400	$ 27,500	$ 38,500
(b) Materials & Services	2,900	3,500	5,000
(c) Indirect Expenses* (1%)	10,200	(2%) 22,200	(2%) 22,700
Total	$ 23,500	$ 53,200	$ 66,200

* 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 FISCAL YEAR 1964

(a) Age Distribution of Cells in Logarithmically Growing Cell Populations. J.R. Cook and T.W. James, chapter in Synchrony in Cell Division and Growth, ed. E. Zeuthen, Interscience Publ., N.Y. (1963).

(b) Techniques for Synchronizing Flagellates. G.M. Padilla and J.R. Cook, ibid.

(c) Ultraviolet-induced Apochlorosis and Photoreactivation in two strains of Euglena gracilis. J.R. Cook. Photochemistry and Photobiology 2: 407 (1963).

(d) Photosynthetic Activity during the Division Cycle in Synchronized Euglena gracilis. J.R. Cook. Journal of Physiology (in press).

(e) Sulfur-containing Nucleotides associated with Cell Division in Synchronized Euglena gracilis. J.R. Cook. Biochimica et Biophysica Acta (in press).

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 cytodifferentiation 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 of 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; Jwan Brachet, Universite Libre de Bruxelles, Belgium; O.H. Scherbaum, Univ. of California, Los Angeles; Lester Goldstein, Univ. of Pennsylvania, Phila.; Vance Tartar, Univ. of Washington, Seattle; Konrad Keck, Johns Hopkins Univ., Baltimore; George Brawerman, College of Physicians and Surgeons, N.Y.

14. TECHNICAL PROGRESS IN FY 1964

1. Nucleo-cytoplasmic interactions during the differentiation of oral structures in Stentor coeruleus

Regeneration of the oral apparatus in the ciliate Stentor coeruleus is accompanied by three striking changes in macronuclear morphology. The

macronucleus normally exists as a chain of nodes, but during regeneration it coalesces into a rounded, irregular mass in the center of the cell then elongates into a rod and finally renodulates. The technique of nuclear transplantation has been used to analyze the regulatory mechanisms involved in bringing about these macronuclear changes. Coalesced macronuclei and macronuclei in various stages of elongation were transferred to non-differentiating cells and to cells in the early phases of regeneration, where nuclear changes have not yet occurred. The experimental results have led to the conclusion that these nuclear changes are regulated entirely by a corresponding series of cytoplasmic changes and not by changes within the nucleus itself. Various properties of the regulatory mechanisms involved in control of nuclear morphology have also been analyzed. Among the findings are evidence that the nucleus cannot respond immediately to the cytoplasmic change initiating its coalescence or complete elongation in the absence of the cytoplasmic conditions which initiate this process.

2. Determination of the Direction of Macronuclear Elongation by a Submicroscopic Organization of the Cytoplasm in Stentor coeruleus.

Experiments in which compacted nuclei were transferred into host cells already possessing compacted nuclei of their own have shown that the direction of macronuclear elongation is determined by a cytoplasmic factor and not by some element of nuclear structure. Stirring the cytoplasm with a micro-needle just before the onset of elongation results in extreme abnormalities of nuclear elongation; highly branched or curved nuclei are produced unless the nucleus is carefully transferred before elongation to a cell in which the cytoplasm has not been stirred. These observations indicate that a submicroscopic organization of the ground cytoplasm exists in Stentor and determines the direction of nuclear elongation. This is especially interesting because electron microscopy of Stentor has not revealed any evidence of submicroscopic orientation in the ground cytoplasm; these results therefore indicate that some cell types possess forms of ultrastructural orientation which may influence morphogenetic events although they are too subtle or too labile to be detectable by electron microscopy.

3. A radioautographic Study of RNA and Protein Synthesis in Stentor coeruleus during Oral Regeneration.

Regeneration of the oral apparatus in the ciliate Stentor coeruleus involves the elaboration of the many thousand cilia comprising the oral membranelles and thus provides an opportunity for studying factors involved in the replacement of a cell organelle composed mainly of protein. One would especially like to know whether regeneration of these cilia is regulated by synthesis of messenger RNA coding for ciliary protein and/or by synthesis of ciliary proteins. Alternatively, regeneration might involve the assembly of pre-existing protein. The extent of incorporation of tritiated uracil and uridine into the nuclear RNA of regenerating and non-regenerating cells was determined by grain counting of the nuclear label in radio-autographs and found to be identical in both groups. Incorporation of tritiated leucine and tyrosine into the newly forming oral apparatus of regenerating cells was also determined by grain counting and found to be negligible. These results strongly suggest that the cilia of the oral membranelles are regenerated by assembly of previously synthesized precursors rather than by de novo synthesis of ciliary proteins.

15. EXPECTED RESULTS IN FY 1965

Investigation of regulatory mechanisms in unicellular organisms such as Stentor and Amoeba will continue. Emphasis will be placed on the following problems: To what extent are the functions of the cell nucleus controlled by feedback of information from the cytoplasm? Why is the cell nucleus immediately required for occurrence of certain cell activities such as regeneration of oral structures in Stentor and intracellular digestion in Amoeba? Is the regeneration of formed cell structures regulated primarily by synthesis of the molecular species composing these structures or by assembly of pre-formed molecules? These problems will be approached by using the techniques of cell micrurgy, radioautography, histochemistry and enzymology as well as by biochemical studies involving the use of radioisotopes.

16. EXPECTED RESULTS IN FY 1966

It is expected that study of the problems discussed in the previous paragraph will continue into 1966. Electron microscopy will be used to determine the early effects of enucleation at the ultrastructural level in Amoeba and Stentor. Techniques will be developed for culturing Stentor in quantities sufficient for biochemical work and fluorescein-labelled antibodies will be prepared against Stentor ciliary protein in order to determine the intracellular location of the precursor pool from which ciliary protein is withdrawn for assembly into membranelles during regeneration of oral structures. Radioautography will be used to ascertain whether protein withdrawn from this pool during regeneration might be replaced during the first hours after completion of regeneration by a compensatory synthesis involving increased production of nuclear RNA.

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 - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
E. H. Strickland

8. Project Term:
From: 1947 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	1	3	3 ½
(b) Other Tech.			
Total	1	3	3 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 10,400	$ 29,700	$ 34,100
(b) Materials & Services	3,000	5,500	7,000
(c) Indirect Expenses*	(1%) 10,200	(2%) 22,200	(2%) 22,700
Total	$ 23,600	$ 57,400	$ 63,800

* 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 1964

None

12. SCOPE OF THE PROJECT

Adenosine triphosphate (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 mitochondrial synthesis 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. Other studies are designed to determine the reactions controlling the rate of ATP turnover in vivo.

13. RELATIONSHIP TO OTHER PROJECTS

Since studies of phosphorylation 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, under Professor David Green at the University of Wisconsin, and under Professor Lester Packer at the University of California.

14. TECHNICAL PROGRESS IN FY 1964

Mitochondria contain significant quantities of non-heme iron. Green and coworkers 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.

Effects of Ferric Nucleotides on Oxidative Phosphorylation

Ferric adenosine diphosphate (Fe(III)ADP) was found to have a number of actions not produced by magnesium-ADP (Mg(II)ADP). Fe(III)ADP appears to be phosphorylated more efficiently than ADP. Fe(III)ADP enhances succinate oxidation to a higher level than does Mg(II)ADP. With pyridine nucleotide-linked substrate, e.g., beta-hydroxybutyrate, malate and glutamate, Fe(III)ADP stimulates mitochondrial respiration only to the same extent as does Mg(II)ADP. The stimulatory action of ferric ADP on succinate oxidation is not specific, as Al^{+++} + ADP, Ba^{++} + ADP, Fe(III) oxalacetate + ADP, and EDTA + ADP are also effective. These results provide further evidence that metals other than Mg(II) may have great influence on oxidative phosphorylation. Further studies are needed to determine the manner in which Fe(III) and other metals affect mitochondrial function.

Interaction of Iron with Mitochondria

Recently evidence has been put forward that mitochondria can accumulate many

divalent cations, e.g., Ca(II), Mg(II), Ba(II), and Mn(II), in an energy-dependent process. Mitochondria may be an intracellular reservoir for a number of cations. In view of the physiological importance of intracellular iron reservoirs, the interaction of iron with mitochondria was investigated. During oxidative phosphorylation using Fe(III)ADP, an appreciable fraction of the iron was found to be bound to the mitochondria. The nature of this iron binding and its energy requirements are being examined with a view to determining the possible physiological importance of mitochondrial iron accumulation.

Digital Computer Representation of Metabolic Pathways

The over-all reaction rate of a metabolic pathway is difficult to evaluate without solving the pertinent kinetic equations. Computations based on a model of the mitochondrial respiratory chain have facilitated interpretation of experimental findings involving effects of ferric chelates. A specific question investigated was whether or not ferric chelates could stimulate succinate oxidation by acting on a reaction in the phosphorylative process without stimulating pyridine nucleotide-linked oxidations. Calculations based on a model system showed that the present data do not permit identifying the particular segment stimulated by iron.

15. EXPECTED RESULTS IN FY 1965

Investigations of the in vitro effects of ferric nucleotides on mitochondrial oxidative phosphorylation will continue. Studies will be initiated to measure phosphorylative reactions in whole cells and perfused organs. Digital computer representations of model metabolic pathways will be carried out when such calculations will aid in interpreting the experimental results.

16. EXPECTED RESULTS IN FY 1966

Attempts to measure oxidative phosphorylation in whole cells and perfused organs will continue. Work will be initiated to determine the possible involvement of the reversal of oxidative phosphorylation in carrying out energy-dependent reactions in vivo. Related investigations will be made to ascertain the mechanism of action of certain ATP-dependent enzymes. Attention will be focused on the importance of the enzyme conformation in the catalytic process.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	3 3/4	4	4
(b) Other Tech.	-	-	-
Total	3 3/4	4	4

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 32,000	$ 39,600	$ 40,700
(b) Materials & Services	2,800	3,000	3,000
(c) Indirect Expenses*	(3%) 30,500	(3%) 33,300	(3%) 34,000
Total	$ 65,300	$ 75,900	$ 77,700

* 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 1964

1. Root transfer of fission products from contaminated soil. Soil Sci. Soc. Amer. Proc. 27: 383-385, 1963.

2. Release and movement of radionuclides in soils contaminated with fall-out material from an underground thermonuclear detonation. U. S. Atomic Energy Commission Report PNE-239F, 1964.

3. Influence of stable Sr and Ca on Sr90 and Ca45 in soils and clay materials. Soil Sci.: Accepted for publication, 1964.

4. Effects of chelates on the movement of fission products through soil columns. Plant and Soil: Submitted for publication, 1964.

12. SCOPE OF THE PROJECT

The objectives of the Soil Factors Section are geared to one of the Divisional objectives of studying the biological cycling of radionuclides released into the environment as a result of application of nuclear energy for various purposes such as power production, excavation, and weapons development. As such, this Section is concerned with soil-plant interrelationship problems. Thus, the work of this Section covers areas of soil and plant sciences. The general objectives of this Section are to determine the factors and the fundamental mechanisms involved in (a) the reactions of radionuclides in soils and plants, (b) the absorption of radionuclides by plants, and (c) the effects of neutron and gamma ray irradiation on soils. These studies are conducted in the laboratory, glasshouse, and controlled plant growth rooms. Under the general objectives, the specific projects that will be pursued during the FY 1965 are discussed below in the "Expected Results" section.

13. RELATIONSHIP TO OTHER PROJECTS

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

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

Hanford Atomic Products Operation, Richland, Washington

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 1964

Experiments were conducted to study the influence of stable Sr and Ca on the behavior of tracer quantities of Sr90 and Ca45 in soils and clay minerals, using an equilibrium batch method. The sorption of tracer

quantities of Sr90 and Ca45 depended on the concentration of the stable Sr and Ca and the kind of soil or clay mineral. Under conditions of low ionic concentration in the equilibrium suspensions, more Sr90 was sorbed in Ca systems than in Sr systems. Under condition of relatively high ionic concentrations, there was no appreciable difference in Sr90 sorption between Ca and Sr systems. Since the initial trace quantities of Sr90 were sorbed firmly the sequence of addition of the tracer to the soil in relation to stable Sr and Ca was important.

Fallout material from an underground thermonuclear detonation was analyzed to determine the presence of several longer lived radionuclides. Fission products that were identified were: Zr95-Nb95, Ru103, Ru106-Rh106, I131, Cs137-Ba137, Ba140-La140, Ce141, and Ce144-Pr144. Other identified radionuclides which probably were produced by neutron activation, were: Sc46, Mn54, Y88, Rh102, W181, W185, and W188-Re188. Radiotungsten was estimated to contribute about 90 per cent of the total gamma activity present in the fallout material 167 days after detonation time.

Studies were made of the influence of distilled water, mock irrigation water, and chelating agents (DTPA, CDTA, and EDDHA) on the movement of Zn65, Sr85 (or Sr90), Y91, Ru106, Cs137, Ce144, and Pm147 in columns of a calcareous soil. Of the radionuclides studied only the movement of Ru106 and radiostrontium was affected by leaching with distilled or mock irrigation water. All treatments moved considerable amounts of Ru106. The chelating agents were only slightly more effective than distilled or mock irrigation water on the movement of Ru106. A 400-inch leaching of the soil columns with distilled water caused about 32 per cent of the applied Sr85 to move. Leaching with the same volume of mock irrigation water caused 99 per cent of the applied Sr90 to move below the zone of contamination. The movement of radiostrontium was related to the presence of Ca ions. Leaching with chelating agents caused no perceptible movement of radiostrontium or Cs137. DTPA and EDDHA caused the movement (58 and 32 per cent, respectively) of the applied Zn65, but CDTA had no effect. Yttrium 91, Ce144, and Pm147 were not moved by distilled or mock irrigation water. CDTA or EDDHA virtually had no effect on the movement of Y91, Ce144, and Pm147; but up to 40 per cent of these ions were moved by DTPA.

15. EXPECTED RESULTS IN FY 1965

(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. Work up to now was done with a calcareous soil. This study will be continued to compare the effects of different soil types - acid, neutral, alkaline-calcareous, and organic soils.

(2) Effects of moisture levels on dissolved quantities of ions in soil solution

The objectives of this project are to study the behavior or tracer amounts of Sr85 and Cs137 in soils as the concentrations and compositions of the soil solution changes. Work with tracer Sr85 was completed and a report is under preparation. Work is continuing to study the reactions of Cs137. This project is conducted in cooperation with Dr. H. A. Hawthorne, Environmental Decay Section.

(3) 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 will be irradiated in UCLA Engineering Reactor and examined for the following: (1) the gross beta and gamma decay rate 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. It is hoped that rapid progress will be made on this project this year. Up to now, progress has been slow, because the reactor has been shut down most of the time for modification.

16. EXPECTED RESULTS IN FY 1966

Certain phases of the projects in progress during FY 1965 will be continued. It is anticipated that the influence of certain chelating agents on the translocation of fission products within the plants will be studied. The project on the effect of neutron irradiation of soils will be continued to study the thermoluminescence of neutron and gamma irradiated soils of different clay mineral types as a function of dose. It is anticipated that a project will be initiated to study certain clay minerals and oxides of metals to determine their adsorptive selectivity for strontium. The objective of this project will be to find means to selectively adsorb radioactive Sr in soils to reduce its uptake by plants.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	4	4	4
(b) Other Tech.	½	½	½
Total	4½	4½	4½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 39,500	$ 43,700	$ 44,800
(b) Materials & Services	2,800	2,800	3,500
(c) Indirect Expenses*	(3%) 30,500	(3%) 33,300	(3%) 22,700
Total	$ 72,800	$ 79,800	$ 71,000

* 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 1964

1. Root transfer of fission products from contaminated soil. Soil Sci. Soc. Amer. Proc. 27: 383-385. 1963.

2. An inexpensive method of reducing the volume of low level radioactive solutions for waste disposal. Soil Sci. Soc. Amer. Proc. 28: (in press).

12. SCOPE OF THE PROJECT

Resources of the Plant Factors Section are directed toward improving understanding of the functions of plants in transferring radioactive contaminants and toxic mineral elements through the soil to the plant link of food chains leading to animals and man. The objectives of this research aim to a better understanding of the fundamental mechanisms involved in plant contamination with fission products and neutron-induced radionuclides. Included are studies of (1) plant contamination with radioactive materials deposited on foliage or absorbed through root systems, (2) the environmental and physiological factors that influence plant uptake and distribution of radionuclides, and (3) the deleterious effects on plants caused by absorbed radionuclides and toxic mineral elements.

Investigations are conducted in controlled laboratory environments and in the field at sites of peaceful application of nuclear energy in order to help bridge the gap between the practical and theoretical problems associated with environmental contamination. It is anticipated that results from this research program will assist in establishing parameters for assessing the consequences to man of radioactive contaminants in the environment.

13. RELATIONSHIP TO OTHER PROJECTS

Research is correlated with other sections of the Environmental Radiation Division:

Soil Factors, Nuclear Medicine & Radiation Biology, H. Nishita

Plant Physiology, Nuclear Medicine & Radiation Biology, W. A. Rhoads

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

Radiation Ecology, Nuclear Medicine & Radiation Biology, W. E. Martin

Related studies are conducted at other on-site laboratories:

Hanford Atomic Products Operation, Richland, Washington

Oak Ridge National Laboratory, Oak Ridge, Tennessee

Los Alamos Scientific Laboratory, Los Alamos, New Mexico

14. TECHNICAL PROGRESS IN FY 1964

Continued studies of the effects of root temperature on fission product uptake by bean and barley plants showed that Sr90, Cs137 and Ru106 uptake from soil and nutrient solution was increased as root temperature was raised from 12° to 32° C. The uptake of Ce144 was decreased by this treatment. Stable Ca, Mg, K and P uptake was increased at the higher root temperatures. Growth of bean and barley plants was inhibited at root temperatures below 12° C and above 42° C. Preliminary results from experiments on the influence of oxygen supply to roots on plant uptake of Sr90 and Cs137 indicated the need to modify certain procedures in order to give more precise control of O_2 concentration and reduce variability in plant growth. Experiments involving the necessary modifications in procedure are in progress.

Barley, bean and alfalfa plants grown on throwout material collected 3500 ft. from the lip of the Sedan crater contained high levels of neutron-induced W181 and W185, and some Mn54 and Sc46. The fission products Sr90, Cs137, Ru106, Rh102 and Ce144 also were absorbed by plant roots and translocated to the foliage. Experiments to further investigate plant uptake and distribution of W185, Ta182, Sc46 and Sb124 from soil and nutrient solution are in progress.

Results of chemical analysis to determine the influence of Be on plant uptake of essential elements showed that the P content increased while the Ca content decreased in tops of alfalfa, barley, lettuce and pea plants grown on nutrient media in which the Be level was raised from 0 to 16 ppm Be. The Na, K and Mg content of plant tissue was not significantly altered by the Be treatments. Similar effects on the content of these essential elements occurred in ladino clover grown on soils treated with Be at levels equivalent to 0, 2, 4, 8 and 16 per cent of the soil cation exchange capacity.

15. EXPECTED RESULTS IN FY 1965

(1) Beryllium effects on plants

The capability of performing routine biochemical analyses of plant tissues was developed during FY 1964. Attention will be directed toward histological studies of Be effects on root development and toward biochemical studies of Be inhibition in plant metabolism. Preliminary studies will include tests on Be inhibition of reactions involved in plant respiration.

(2) Influence of oxygen supply to roots on plant uptake of radionuclides

Studies will continue in the investigation of the influence of changes in concentration of O_2 and CO_2 in the root environment on the absorption and distribution of radionuclides by higher plants, and to investigate the changes in plant metabolism caused by altering the CO_2 and O_2 concentration of the root environment. Some phases of this project will involve collaborative work with the Plant Physiology Section.

(3) Accumulation of fission products and neutron-induced radionuclides by crop plants

Investigation will continue on plant uptake of neutron-induced radionuclides and fission products from contaminated soils. Emphasis will be placed on experiments to investigate the reactions of neutron-induced radionuclides in soils and clay systems, including the fixation, exchange and mobility of W185, Ta182, Sb124 and Sc46. Some phases of this project may involve collaborative work with the Radiation Ecology Sections in areas of peaceful application of nuclear energy.

16. EXPECTED RESULTS IN FY 1966

We anticipate that research in progress in FY 1965 will be continued during FY 1966. Emphasis will be placed on studies involving neutron-induced radionuclides. During FY 1966 we expect to have developed the capability to initiate studies on the effects of irradiation on some of the native plant species from the NTS study areas in our controlled plant growth facilities. This work will be corroborative to plant irradiation studies at the NTS Ecology study area.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	6	5½	5½
(b) Other Tech.	-	½	½
Total	6	6	6

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 51,700	$ 59,500	$ 61,700
(b) Materials & Services	11,000	12,300	12,300
(c) Indirect Expenses*	(5%) 50,800	(4%) 44,400	(4%) 45,300
Total	$ 113,500	$ 116,200	$ 119,300

* 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 1964

1. A visual null-point indicator for a soil moisture bridge. Agron. Jour. 56 (2): (in press).

2. An inexpensive resistance welder for thermocouple fabrication. Ecology 45, (submitted for publication).

12. SCOPE OF THE PROJECT

The purpose of the farm study is to obtain data for the construction of stochastic models which quantitatively describe the Sr90, Cs137, and Ce144 cycles within the farm. The quantity of each fission product present, the proportion of each that is biologically active, and the statistical variation in concentration of isotopes is determined for each component of the farm system.

Small numbers of samples from each farm component are analyzed radiochemically. Data about the variation in radionuclide composition of these samples is used to compute the numbers of additional samples required to establish statistically valid sampling and radiochemical programs. Laboratory studies are conducted utilizing plants and soil from the farm under standardized growing conditions to obtain bases for assessing plant uptake of fission products.

The results from the combined field and laboratory studies of this project provide a means of interpreting and of utilizing the great mass of data which has accumulated about the occurrence of fallout in human food chains over the earth. The fission product models developed here will enable the extreme concentrations of fission products, as well as the averages, to be predicted realistically. This is of major import when predicting the proportion of exposed populations that may acquire hazardous levels of radionuclides from foods produced after different kinds of contaminating events.

13. RELATIONSHIP TO OTHER PROJECTS

Similar studies of fission product cycling in dairy farms are in progress at Colorado State University and at University of Michigan, Ann Arbor, Michigan. Collaborative studies are conducted within the Laboratory of Nuclear Medicine and Radiation Biology with the Soil Factors Section and the Desert Ecology Section.

14. TECHNICAL PROGRESS IN FY 1964

During FY 1964 collection of samples in the field required 200 man-days, excluding preparation of samples for storage. In the dairy study nine feeding trials of 10 days duration were undertaken to measure the biological availability of fission products associated with different species of cultivated plants. Materials were collected from each of 10 crops harvested as part of the farm operation. Fallout collectors were operated during the growing period of each crop, incoming irrigation water, pre-

cipitation, runoff water from the irrigations and one rainstorm were sampled. Silt was filtered from the irrigation water, collected from the concrete irrigation canal and the drainage water system and from the lower portion of cultivated fields. To accomplish the multiple objectives of this study 24 distinct kinds of samples are required. Alfalfa plants were brought to the laboratory in the soil they were growing in for cropping under reproducible conditions. Eight crops of alfalfa were produced between July 1 and January 4 compared to three at the farm.

Preliminary data from five feeding trials conducted in the dairy suggest different concentrations of Sr90, Cs137, and Ca exist in morning milk compared to night milk. The statistical validity of the difference seems to rest upon a few values among the 99 samples. Additional analyses are in progress to determine if these samples fall outside normal sample variation. Variances within-feeding-trials were greater than between-feeding-trials, were not statistically significant for Sr90 and Ca, apparently were significant for Cs137 with the feeding of silage in midmorning versus midafternoon.

Groups of six samples of different kinds of plant materials were analyzed radiochemically to evaluate the variance present. Standard errors were six per cent for Sr90 and 14 per cent for Cs137 and for Ce-Pr144. The average standard error in plant yield from a square meter of field alfalfa was five per cent in four crops. It appears that the desired standard error of six per cent can be achieved economically for feeds produced on the farm. Radionuclide concentrations of 1962 alfalfa were lowest in midsummer and higher in early summer and late summer. Sr90 and CS137 levels were similar in all alfalfa crops but the Cs137 levels of silage produced in July through October was twice that of Sr90. Preliminary data from a test for contamination of plants by radioactive soil indicated less than one per cent of the Sr90 associated with 1962 alfalfa samples was due to soil.

A data reduction program for computation of sample fission product concentrations was initiated for an IBM 7090 computer. The variance for different isotopes is computed on groups of samples in order that Sequential Analysis techniques may be used to forecast the numbers of samples needed to achieve reliable statistical precision.

Collaborative work with the Soil Factors Section was completed for the evaluation of the effect of soil moisture tension on the solubility of Sr85 in soil solution and a manuscript is in preparation. A similar study is in progress using Cs137. Studies of the relationship between periods of soil moisture deficit and the development of desert annuals was begun at the Nevada Test Site in cooperation with the Desert Ecology Section. This study will be correlated with biochemical studies when data about plant development are obtained.

15. EXPECTED RESULTS IN FY 1965

Stochastic models of fission product cycles and the interchange of radionuclides will continue to be the major endeavour. The initial models will be developed on a deterministic basis with the assumption that the variation in fission product concentrations has a normally distributed curve. When sufficient data are available the actual distribution will be fitted to Pearson Type III frequency distributions for each fission product. The necessary scale and shape parameters for the observed distributions will be calculated from a recently developed maximum likelihood approximation and isotope concentration probabilities will be derived for each component of the farm system. Stochastic models can then be developed using input data from soil and plant studies which utilized soil and plants from the farm concurrently with those from fourteen other locations in California and Nevada. Integration of laboratory data with field data adds enormous power to the predictions possible.

The present computer program for calculation of variances, standard errors and standard deviations, for factorial analysis of batteries of data will be extended to handle calculations for the various models as they are developed. This will require the assistance of a competent programmer and occasional access to a professional statistician.

Laboratory studies of the effect of soil moisture tension upon the release of Cs137 from soil to soil solution will continue. Exploratory work to develop a quantitative test of past exposure of plants to periods of high moisture stress will progress as work for the farm program permits. Data from soil moisture and soil temperature studies at the Nevada Test Site will be correlated with phenological observations acquired at the same sites.

16. EXPECTED RESULTS IN FY 1966

Most of the facilities will be utilized in phase-out operations connected with the close of the farm program. Preparation of samples for radiochemical processing will extend through this Fiscal Year and into the next one. Data from work in Fiscal Year 1965 will be used to reinforce models in development with emphasis upon the probabilistic aspects which will require the addition of a technician trained in mathematics.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	2	3	3
(b) Other Tech.	½	-	-
Total	2½	3	3

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 19,800	$ 30,800	$ 33,000
(b) Materials & Services	2,100	2,100	2,800
(c) Indirect Expenses*	(2%) 20,300	(2%) 22,200	(2%) 22,700
Total	$ 42,200	$ 55,100	$ 58,500

* 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 1964

None

12. SCOPE OF THE PROJECT

This project purposes to investigate the fundamental physiology of the roots of plants under conditions more nearly like those found in the environment of plant roots, 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 or root metabolism is known by analogy to other plant parts, or to other metabolic systems, both largely from cytoplasmic particles, isolated and in vitro. The objective of this project is thus to investigate the respiratory rates and metabolic processes of intact roots under conditions of varied environments. The first objective is to investigate the effects of increased CO_2 concentrations about the roots, to find whether the greatly 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.

Methods of studying the gaseous exchange about roots have been developed which utilize both volumetric analysis and infra-red 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 investigated in the laboratory. This allows investigation of a variety of conditons of the CO_2 - O_2 environments of roots, and also an investigation of the interaction of varied mineral nutrient concentrations with changes in CO_2 - O_2 concentrations. This should provide information regarding mineral uptake, or radionuclide uptake, by plant roots.

The method used by this project is to grow a species or a variety of species of plants in Hoaglands solution and aerate it with gases of known concentrations. By monitoring the concentrations of the aerating gases before entering the root media and after leaving it, respiration rates can be determined; and by utilizing carbon-14 in the aerating gases, the path of carbon utilization can be followed. By using radioactive isotopes of those minerals utilized by plants much can be learned of the effects on absorption and translocation under the conditions of the study. This will contribute to understanding of the uptake of radiostrontium and -cesium by plants.

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, U. C. L. A.

Department of Hort. Sci. U. of California, Davis, California

14. TECHNICAL PROGRESS IN FY 1964

Bean plants were grown in growth chambers in containers arranged so that the aerating gases entering and leaving the nutrient solution could be monitored either by infra-red spectrometry, by a Burrell Gas Analysis apparatus or by a Beckman Oxygen analyzer. Aerating gases consisting of air with from 2% to 20% CO_2 added were used. With all concentrations tested, bean plants grew quite well; and the changes in the concentrations of CO_2 after contact with the root media were small, and of the same magnitude regardless of the initial concentration in the aerating gases. Further analysis are under way.

Analysis of small changes in high concentrations of CO_2 is a difficult task, and for this purpose a special chamber with sapphire windows and having a light-path length of 1 cm was designed and built. Further refinements are in progress.

15. EXPECTED RESULTS IN FY 1965

Considerable knowledge concerning the responses of several species of plants, known to be adapted to extremes in aeration to roots will be achieved. The reactions of plants known to be capable of fixing CO_2 in the dark in leaves will be investigated to determine whether this capacity may exist in roots also. Such knowledge is potentially of great importance to understanding metabolic processes of plants growing in calcareous regions, and in contrast, of those only capable of growing in acidic soils. This should yield important information concerning the utilization of iron, particularly, and many other mineral elements and fission products. The application of this knowledge to the ecology studies of the Nevada Test Site is proposed.

16. EXPECTED RESULTS IN FY 1966

By this year results should be available not only on the metabolic processes of various species of plants but on the concommitant mineral nutrition of these plants also. These experiments will contribute to the understanding of the utilization of, and concentrations of, C^{14} in plants from C^{14} in the atmosphere as the result of both natural causes and from nuclear events of any origin.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	5	6	6
(b) Other Tech.	-	-	½
Total	5	6	6½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 46,000	$ 59,400	$ 62,700
(b) Materials & Services	4,900	6,500	10,600
(c) Indirect Expenses*	(4%) 40,600	(4%) 44,400	(4%) 45,400
Total	$ 91,500	$110,300	$118,700

* 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 1964

1. Fallout and natural populations, pp. 152-156, Proceedings First International Conference on Wildlife Disease. Published July 1963.

2. Analysis of reproduction in a black-tailed jack rabbit population. Proceedings XVI International Congress of Zoology 1: 258.

12. SCOPE OF THE PROJECT

The objective is to investigate the effects of ionizing radiation on populations of animals. This approach treats the population as the functional unit, and examines such characteristics of the population as the intrinsic rate of increase and age structure.

Laboratory studies utilize a wild species of mouse Peromyscus maniculatus. Animals are living under chronic exposure to low level radiation. Their reproductive performance will be compared to reproductive performance of a control group.

A field experiment is in progress at the Nevada Test Site. A natural population of rodents is exposed to chronic low level radiation. The population is censused at monthly intervals. A life table for these animals will be constructed and compared to a life table for the control population.

The results should indicate the level of chronic exposure that can be tolerated by a population of rodents. It is assumed that a certain fraction of the population can be lost, due to effects of radiation or other causes, and that the population can still maintain itself. If it fails to maintain adequate numbers, either by excessive loss or reduced production, we will observe the mechanism of action.

13. RELATIONSHIP TO OTHER PROJECTS

Under simultaneous investigation in the irradiated field study plot are the plant population and the reptile population by other investigations from the Laboratory. Plans are being formulated to include invertebrates as well. Other population studies are being conducted at the Oak Ridge and Savannah River installations. Effects of radiation on laboratory populations are being investigated at Los Alamos Scientific Laboratory, Texas Engineering Experimental Station, University of Wisconsin, Department of Zoology at Oxford, and the Radiobiology Research Unit at Harwell.

14. TECHNICAL PROGRESS IN FY 1964

1. Field Studies in Rock Valley at the Nevada Test Site have continued. A radioactive source of cesium-137 has been installed in one study area, providing a dose rate at ground level of 3 to 5 r/day in 60% of the study area, and 2 to 3 r/day in 30% of the study area.

2. From a breeding stock of Peromyscus maniculatus in the laboratory, 30 pairs of irradiated animals and 30 pairs of controls have been established.

The offspring of the irradiated group are being combined to provide the study group which will actually be composed of animals that have been irradiated since conception. Based upon observed variability in this species, with 30 irradiated pairs and 30 control pairs there will be an 80% chance of showing a difference of 10% in the reproductive capacity of the two groups.

3. Effort has continued on a new method for age estimation of small mammals in the field. Measurement of the uptake of radioisotopes by the dentin in sections of teeth appears to provide a good correlation with age.

15. EXPECTED RESULTS IN FY 1965

1. The radioisotope method for age estimation of rodents collected in the field will be perfected for routine use. As this is a new method that has not been utilized before, it should be reported. The method will be applied to specimens from Rock Valley that were collected before radiation studies began. This will indicate the age structure of the population, which when combined with the data on population density will provide the ingredients for a life table. The life table tells mortality rates and life expectancy for intervals of age. This type of information has never been produced for a population of desert rodents, and is available for only a few natural populations of mammals.

2. Examination of ovaries for recent corpora lutea and of uteri for placental scars from specimens collected in Rock Valley prior to the radiation study will reveal the age-specific fertility of the population. This, when added to the life table, is the data required for evaluation of the capacity for increase of the population. This evaluation will be made and will be available for comparison with the enclosed population and the irradiated population.

3. Census of the mammal population in each of four study areas in Rock Valley at the Nevada Test Site will be continued at monthly intervals. One week is devoted to each area. Census is accomplished by operating four hundred mammal traps for three successive nights and recording the presence, location, and condition of each animal. Animals are marked by a numbering system for individual recognition.

16. EXPECTED RESULTS IN FY 1966

1. A full years study of the irradiated mammal population at the Nevada Test Site will be completed, and the results of two breeding seasons will be available. The data should be ready for analysis and perhaps a tentative statement of radiation effects on the population can be made.

2. The experimental pairs of deer mice, Peromyscus maniculatus, maintained in the laboratory under conditions of irradiation similar to the field study, will provide data on the fertility of this species for comparison to control animals in the laboratory and to the populations in the field. The animals are provided with shelters which contain small sealed sources of cesium-137. Each animal receives approximately 1 r/day throughout life. The life time fertility of these animals is to be compared to a control group.

3. Evaluation of energy flow in the ecosystem of Rock Valley at the Nevada Test Site will begin by measuring the metabolic rate and energy utilization of some of the animals. Metabolic rate under different climatic conditions will be measured by use of an oxygen analyzer and a controlled temperature cabinet. Energy requirements and utilization will be estimated from estimates of caloric content of the food supply and the animal population. Eventually such techniques should be applied to each trophic level of the ecosystem.

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 Nevada Test Site

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

4. Date Prepared:
April - 1964

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

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Janice C. Beatley

8. Project Term:
From: 1961 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	3 3/4	4	4
(b) Other Tech.	1	1	1
Total	4 3/4	5	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 36,400	$ 45,100	$ 47,300
(b) Materials & Services	7,000	7,600	7,600
(c) Indirect Expenses*	(3%) 30,500	(3%) 33,300	(3%) 34,000
Total	$ 73,900	$ 86,000	$ 88,900

* 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

1. Annual vegetation of the northern Mojave Desert. Bull. Ecol. Soc. Amer. 44: 122-123. 1963 (Abstract)

2. Vegetation and environments of the Nevada Test Site. Bull. Ecol. Soc. Amer. 44: 123. 1963 (Abstract)

3. The sunflowers (genus Helianthus) in Tennessee. Jour. Tenn. Acad. Sci. 38: 135-154. 1963

4. Vegetational analyses of desert shrub communities of the Nevada Test Site, Nye County, Nevada (with William H. Rickard). In press.

5. Post-Sedan one and one-half years: Effects on desert vegetation of a nuclear detonation, Nevada Test Site. Submitted for publications, February, 1964.

12. SCOPE OF THE PROJECT

Objectives are (1) to establish biological and environmental-effects baselines exhibited in the diverse types of vegetation in all parts of the Test Site, so that the effects of environmental change on biological systems associated with Test Site testing activities may be detected and evaluated; and (2) to determine what may be the effects, if any, of ionizing radiation from nuclear testing at the Test Site upon the native vegetation.

13. RELATIONSHIP TO OTHER PROJECTS

Radioecological studies conducted on other AEC-DBM contracts, especially Brookhaven National Laboratory, Oak Ridge National Laboratory, and Emory University, and desert biological research conducted in various institutions, have indirect or direct relationship to the Test Site ecological problems.

14. TECHNICAL PROGRESS IN FY 1964

(1) Vegetation studies

On the 67 permanent study plots established in the spring of 1962, phytosociological data have been collected at the appropriate seasons for (a) shrubs, (b) herbaceous perennials, (c) annuals, and (d) seedlings of all species. A seedling collection, enabling identification of most vascular species at this stage, was made. A computer program has been devised for analyses of the shrub data, and the data recorded on IBM punch cards. Biomass data for a segment of the vegetation will be obtained in the spring of 1964.

(2) Environmental measurements

On the permanent study plots, weekly records of precipitation, maximum

and minimum air temperatures, and ionizing radiation have been obtained since November, 1962.

Soil moisture blocks and thermocouples were installed in the autumn of 1963 in the root zone of annuals on all plots. The choice of moisture sensors suitable for NTS soils and moisture regimes, their calibration over a range of moisture tensions, and the installation of moisture and temperature sensors have been a 3-year cooperative research project with Dr. Howard A. Hawthorne.

Mechanical analyses of soils from all plots, and certain other soil characterizations, are in progress.

(3) Radiation effects

(A) The Larrea radiation effects study, in progress for several years, will be completed with (a) the data for a second year for germination percentages of seeds from NTS populations which have been irradiated by nuclear testing and populations which have not been exposed to increased levels of radiation from test activities, and (b) comparable data for seeds irradiated at 3 levels with the Cobalt source at the Laboratory.

(B) Quantitative and qualitative data have been obtained for vegetational phenomena of the permanent study plots located in the Sedan area where (a) above-ground parts of shrubs were destroyed by the blast, (b) shrubs were partially uprooted by the blast, and (c) there was no blast damage but, like the other areas, there was deposition of a blanket of radioactive dust.

The expression of these phenomena has been compared with that of these same species elsewhere on the Test Site.

(C) A third Pinyon Pine population was monitored at the time of detonation of an underground device on Ranier Mesa, in the event of significant dosages of ionizing radiation from venting. Dosages were less than 100 mr.

(4) NTS Herbarium

Processing of the backlog of plant specimens was completed, and those of certain groups were sent to specialists for review.

15. EXPECTED RESULTS IN FY 1965

Vegetation and environmental measurements will continue, be recorded on IBM punch cards, and during the year computer correlation analyses made between the biological and environmental variables. These will be the basis of a major treatise on the vegetation and environments of the Test Site.

Radiation effects studies will continue in the Sedan area. These will be both field and laboratory studies of vegetative and reproductive characters

of shrub and herbaceous species in all areas of Yucca Flat, designed to detect possible effects of the radiation in the environments of these species in the Sedan area of northern Yucca Flat.

Against the background of information about the species of the Test Site and their environments, which will have accumulated by this time, several kinds of field biological studies will be conducted at the appropriate seasons, and others employing materials obtained from some or all of the permanent study sites will be conducted at the Laboratory.

16. EXPECTED RESULTS IN FY 1966

Since most field studies under way are long-term in nature, these will continue through this fiscal year. Laboratory studies will continue to be conducted in support of the field problems, many of which have been defined but will require experimental laboratory work for their solution. No physical expansion in any project 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.:	4. Date Prepared:
06-05-01	April - 1964
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: Frederick B. Turner	8. Project Term: From: 1962 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2	2½	2½
(b) Other Tech.	½	-	-
Total	2½	2½	2½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 19,300	$ 25,300	$ 27,500
(b) Materials & Services	6,200	3,000	3,600
(c) Indirect Expenses*	(1%) 10,200	(2%) 22,200	(2%) 22,700
Total	$ 35,700	$ 50,500	$ 53,800

* 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 1964

1. Biotic communities of the Nevada Test Site (review). Ecology 44: 633-634.

12. SCOPE OF THE PROJECT

The effects of long-term low-level chronic radiation on natural populations of animals are not known. Such information is of value in understanding the influence of environmental radiation on natural communities and estimating its possible effects on man.

The objective of this study is to define the effects, if any, of continuous exposure to low levels of gamma radiation on populations of lizards within two 20-acre enclosures. The premise is adopted that the effects of irradiation will be manifested in a decline in numbers of individuals. This decline, if it occurs, is to be documented and the possible causes are to be investigated. The study will be carried out over a period of at least three years and will involve comparisons of populations of lizards (Cnemidophorus tigris and Crotaphytus wislizeni) existing within enclosures in Rock Valley. Experimental areas will be exposed to continuous gamma irradiation from a Cs-137 source. One fenced enclosure and one unfenced area will not be irradiated and will serve as controls. The demographic data will be drawn in a series of samples taken by hand and with traps. Traps within the enclosure will be arranged in a 15-meter grid (about 400 traps in each enclosure). Trapped animals will be weighed, measured, marked, and released at point of capture. The age-distributions and sex-ratios of the populations will be estimated from the trap samples. Fertility will be estimated from the numbers of breeding females and young-of-the-year. These data will reveal any significant differences in survival-rates in the experimental and control areas. Any significant changes in the total numbers of individuals, or in the age-distributions of the populations will also be apparent. The estimates of fertility will expose any changes in the reproductive potential of irradiated females.

13. RELATIONSHIP TO OTHER PROJECTS

This program is being developed in Rock Valley, using 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.

14. TECHNICAL PROGRESS IN FY 1964

Following the fall of 1963, an evaluation of sampling data from the fenced control area in Rock Valley indicated that the leopard lizard (Crotaphytus wislizeni) was affording more useful information than the side-blotched lizard (Uta stansburiana). Accordingly, whiptailed lizards (Cnemidophorus tigris) and leopard lizards became the species of primary interest. Seasonal incidence of the two species during 1963 is indicated below:

	March	April	May	June	July	August	September	October
Cnemidophorus tigris	0	7	36	41	51	62	7	0
Crotaphytus wislizeni	5	11	12	8	4	6	1	1

These numbers reflect all individuals registered during each monthly interval. By the end of May, all of the leopard lizards had apparently been recorded, and by the middle of August almost all of the whiptailed lizards within the enclosure had been captured at least once. The size and composition of the two populations (as of April 1963) were estimated as follows:

	Adult males	Adult females	Subadult males	Subadult females	Totals	Density per acre
Cnemidophorus	33	24	25	29	111	5.55+
Crotaphytus	2	3	8	8	21	1.05+

Reproduction during 1963 appeared to be very low. Only four young whip-tailed lizards and two young leopard lizards were taken during the latter part of the 1963 season. Reproduction by small mammals in Rock Valley was also poor in 1963.

On October 14, 100 juvenile Uta stansburiana were irradiated with a Co^{60} source. Doses were administered at 91 r/minute to groups of 10 individuals. The doses ranged from 635 to 1450 r. During the following 4 months the survival and growth of the 100 irradiated animals was as good as that of 60 controls kept under the same conditions. It is concluded that the $LD_{50,30}$ for this species is more than 1450 r, a finding not in agreement with work at Texas Technological College where the $LD_{50,30}$ was estimated to lie between 900 and 1200 r.

15. EXPECTED RESULTS IN FY 1965

Sampling will be expanded to include four 20-acre areas, one of which is continuously irradiated except for a 4-day period each month. Each area will be sampled monthly, for a period of four days. Adult female lizards will be collected in adjoining parts of Rock Valley in order to determine the ovarian cycle and to estimate size-specific fertility by counts of ovarian and oviducal eggs.

Experiments involving acute irradiation will be continued. The possible influence of age will be explored. The survivors of the first experiment will be irradiated again in order to see if their resistance has been impaired. Experiments with whiptailed lizards are planned.

16. EXPECTED RESULTS IN FY 1966

Sampling of the four study areas will be continued. Attempts will be made to analyze populations of Uta stansburiana as well as of whiptailed and leopard lizards. The general pattern of size-specific fertility in these species should be apparent by this time, as well as the survival curves for adult individuals. The radiosensitivity of three species of lizards will have been defined, at least for acute gamma exposures. Studies of the nuclear volumes of cells of these species will be undertaken and these findings correlated with acute radiosensitivity.

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 Studies

3. AEC Budget Activity No.:	4. Date Prepared:
06-05-01	April - 1964
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: 1962 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2	2½	2½
(b) Other Tech.	-	-	-
Total	2	2½	2½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 18,700	$ 26,400	$ 27,500
(b) Materials & Services	5,600	3,000	3,500
(c) Indirect Expenses*	(1%) 10,200	(2%) 22,200	(2%) 22,700
Total	$ 34,500	$ 51,600	$ 53,700

* 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 1964

None

12. SCOPE OF THE PROJECT

The purpose of this project is to investigate the effects of nuclear radiation on natural plant populations. Specifically, it concerns the effects of chronic exposure to low levels of gamma irradiation from a cesium-137 source upon populations of desert plants in their natural environments. The field facilities being utilized for this study are the same as those established for Dr. N. R. French's study of radiation effects on small mammal populations in Rock Valley at the Nevada Test Site.

The exposure of plants in their natural environments to high levels of chronic gamma radiation (generally $>$ 30 r/day) has been shown to have marked influences on the growth, development, reproduction, and physiological tolerances of plants to environmental factors other than ionizing radiation. Chronic exposure to relatively low levels (2-4 r/day) of gamma radiation may or may not elicit measurable responses. Because of this uncertainty, our primary objective is to compare the seasonal behavior of irradiated plant populations with that of non-irradiated populations. If significant differences are observed, we shall attempt to determine whether those differences are related to gamma radiation or to some other environmental factor.

The Rock Valley study area consists of 4 circular plots each having an area of about 20 acres. Three of these plots are enclosed by rodent-proof fences, and one of the enclosures has been equipped (January 1964) with a partially-shielded gamma source (Cs-137). The three non-irradiated plots (2 fenced and 1 unfenced) will serve as controls.

Comparison between the plant populations of the irradiated plot with those of the non-irradiated plots will be based on (1) measurements of environmental factors such as air and soil temperature, humidity, precipitation and soil moisture, (2) estimates of plant population parameters such as density, frequency, and dominance of the species present, and (3) measurements of growth of photosynthetic tissue and estimates of reproductive success based on flower and seed production, seed germinability and seedling survival.

Due to the non-uniformity of the gamma field, it is also desirable to measure gamma doses to which plants in different parts of the irradiated plots are exposed. The cytological and anatomical characteristics of irradiated and non-irradiated plants are also of interest and will be investigated. If feasible, studies of productivity and the turnover of mineral nutrients may also be conducted in or near the Rock Valley study plots.

13. RELATIONSHIP TO OTHER PROJECTS

These studies are closely related to studies being conducted in the same area by Drs. N. R. French and F. B. Turner of this Laboratory. Similar studies of plant community structure and composition in relation to microclimatic and edaphic factors are being conducted at the Nevada Test Site by Drs. J. C. Beatley and H. A. Hawthorne, also of this Laboratory. Similar studies of the effects of ionizing radiation on natural plant populations 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 1964

Preliminary studies were made to characterize the soils and plant populations of the Rock Valley study area. The soil survey indicated two principal soil types. Desert pavement, which is virtually devoid of vegetation, is characterized by a smooth surface layer of stones embedded in a vesicular layer of silty loam. Young alluvium is characterized by an irregular surface of stones and no vesicular layer. In both cases the subsoil is stoney and characterized by caliche deposits. The species composition of shrub vegetation was found to vary from plot to plot, but statistical analyses indicate no significant differences in regard to the importance values of the four leading species (Franseria dumosa, Larrea divaricata, Ephedra nevadensis, and Lycium andersonii) which accounted for more than 66% of the 1600 plants included in population samples. Nuclear volume data, provided by Dr. N. F. French, indicate that Ephedra nevadensis may be the most radiosensitive species present in the gamma field.

15. EXPECTED RESULTS IN FY 1965

Instruments installed during the second half of FY 1964 should provide data concerning seasonal variations of maximum and minimum air temperature, soil temperature, precipitation, and soil moisture at six points in each of the four plots. If feasible, data will be also collected to estimate gamma exposure doses to plants in different parts of the gamma field.

Observations and measurements begun during the second half of FY 1964 and continued periodically through FY 1965 should provide data for the comparison of plant population behavior in the irradiated plot with that of similar populations in the non-irradiated plots. These will include estimates of photosynthetic tissue production and of reproductive success as outlined earlier.

16. EXPECTED RESULTS IN FY 1966

Procedures followed in FY 1964 and FY 1965 will be continued. Plans are being made for data reduction by means of existing computer programs.

By FY 1966 it would be desirable to supplement and expand radiation botany studies at the Nevada Test Site to include the effects of high level, chronic irradiation on plant populations similar to those in the

present Rock Valley study area. This would require a semi-portable unshielded radiation source which could be raised and lowered by remote control. Ten kilocuries of Cs-137, for example, raised to a height of 15 ft. would expose a circular area of about 6 acres, containing 6000 to 7500 shrubs, to chronic radiation doses ranging from >1000 to $<$ /r/day. This would provide sufficient replication of most species to provide good estimates of the major parameters relating to the relative radiosensitivities of various species. After a suitable period of exposure, the source could be moved to another community type, and similar estimates would be obtained for a different array of species. In this manner it should be possible to obtain a great deal of comparative data in an efficient and economical manner.

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

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

4. Date Prepared:
April - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	5	5	5
(b) Other Tech.	-	-	-
Total	5	5	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 43,700	$ 47,500	$ 50,800
(b) Materials & Services	12,900	11,900	13,600
(c) Indirect Expenses* (3%)	30,500	(3%) 33,300	(3%) 34,000
Total	$ 87,100	$ 92,700	$ 98,400

* 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

Publications of the work of this Section appear under the various sections.

12. SCOPE OF THE PROJECT

The basic purpose of the Chemical Problems Section is to maintain and operate a radiochemical and analytical facility in order to support the various program objectives of the fallout study work of the Environmental Radiation Division. To this end an efficient laboratory capable of handling routinely relatively large numbers of low-level radioactive samples is maintained. During FY 1964 there was continued interest in radiostrontium-89, -90, Cs-137, Cs-Pr-144, and Ba-La-140.

Analytical techniques include the use of a 512-channel pulse height analyzer particularly for analyses of neutron-activated soils. In this work, Na-24, W-181, and Mn-54 are the important radionuclides usually encountered. Plant and animal tissues having incorporated these nuclides are also subjects of analysis.

This Section also assists other sections in the preparation and standardization of isotopic solutions and in the calibration of working standards. The Section carries on research into methods of analysis; and with the appointment of a new Section Chief, will expand this research endeavour into radiochemical research beyond that of support for the other sections of the Environmental Radiation Division.

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 & 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, Tennessee
All other Sections of Environmental Radiation Division
U.S. Naval Radiological Defense Laboratory
Lawrence Radiation Laboratory

14. TECHNICAL PROGRESS IN FY 1964

The following projects received support from this Section.

Environmental Decay Section

Four thousand five hundred analyses were processed during FY 1964. These were for Sr89, -90, Cs-137, Ce-144, Ra-226, stable Sr, K, P, and Ca. These determinations were made on soil, irrigation water, milk, crop material, feeds, feces, and G. I. tract material.

Soil-Plant Studies

Two hundred and fifty analyses were completed for Pu, gross rare earths, gross alkali metals, and gross alkaline earths.

Radiation Ecology

100 analyses of deer-jaws for radiostrontium were made.

This Section has assisted in the development of a computer analysis program designed to simplify the task of data reduction. This appears ro be a pioneer effort in the use of computers in this particular area, i.e. to do the multiplicity of calculations necessary in the field of radiochemistry. This work is an important step toward a more intensive use of computers in radiochemical counting and analysis.

One improvement in the chemical procedure for analysis of soil for both Cs and Ce was developed. A manuscript covering this is in preparation.

15. EXPECTED RESULTS IN FY 1965

It is anticipated that support to the Soil Factors Section will necessitate the processing of a large number of neutron-activated soils, both by gamma spectrometry and radiochemistry. This work will depend, however, on continued operation of the UCLA reactor as a source.

With the appointment of a Section Chief it is expected that original research activities will augment the heretofore largely supporting activities of this Section.

Support to the Environmental Decay Section will continue on a large scale since the farm program of that Section will be one year from its scheduled termination date.

16. EXPECTED RESULTS IN FY 1966

Continued support of the other sections in the Environmental Radiation Division is anticipated. The nature of this support will be governed by the scope and objectives of these sections.

More research originating within this Section is anticipated. The appointment of a Section Chief during FY 1965, if consummated, will broaden the scope of research into problems in radiochemistry and geochemistry in keeping with the needs of the Commission.

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 Studies

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

4. Date Prepared:
April - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	5	5	5
(b) Other Tech.	-	-	-
Total	5	5	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 38,500	$ 40,700	$ 41,800
(b) Materials & Services	11,100	9,900	12,400
(c) Indirect Expenses*	(3%) 30,500	(3%) 33,300	(3%) 34,000
Total	$ 80,100	$ 83,900	$ 88,200

* 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 1964

Ten publications of the work of this Section appear under the following Projects:

Nuclear-Events-Biological Studies

Environmental Assessment - Plant Study

12. SCOPE OF THE PROJECT

The basic purpose of the Chemical Problems Section is to maintain and operate a radiochemical and analytical facility in order to support the various program objectives of the fallout study work of the Environmental Radiation Division. To this end an efficient laboratory capable of handling routinely relatively large numbers of low-level radioactive samples is maintained. During FY 1964 there was continued interest in radiostrontium-89, -90, Cs-137, Cs-Pr-144, and Ba-La-140.

Analytical techniques include the use of a 512-channel pulse height analyzer particularly for analyses of neutron-activated soils. In this work, Na-24, W-181, and Mn-54 are the important radionuclides usually encountered. Plant and animal tissues having incorporated these nuclides are also subjects of analysis.

This Section also assists other sections in the preparation and standardization of isotopic solutions and in the calibration of working standards. The Section carries on research into methods of analysis; and with the appointment of a new Section Chief, will expand this research endeavour into radiochemical research beyond that of support for the other sections of the Environmental Radiation Division.

13. RELATIONSHIP TO OTHER PROJECTS

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, Tennessee

All other sections of Environmental Radiation Division

U.S. Naval Radiological Defense Laboratory

Lawrence Radiation Laboratory

14. TECHNICAL PROGRESS IN FY 1964

The following projects received support from this Section in FY 1964.

Fallout and Environmental Studies

(1) Strontium 89 and Sr-90 analysis were completed on approximately 250 bone samples. In addition Sr-89 and Sr-90, Cs-137, stable Ca, Mg, Si, and Fe analyses were completed on soil, muscle, G.I. tracts and plant samples. A total of 1385 determinations were completed to support these research projects.

(2) The pot collection program initiated in 1960 continued to be supported by this Section.

Miscellaneous Studies

This Section continued to assist other AEC agencies and contracts in cross-checking radiochemical analytical procedures for various radionuclides.

15. EXPECTED RESULTS IN FY 1965

The Section anticipates at least 5000 determinations will be completed during the next fiscal year in support of other sections. It anticipates an increase in analyses associated with original research within the Section. The 512-channel gamma spectrometer has been in full operation during FY 1964; and, as of March 1964, several projects are being supported by work from this instrument with an anticipated increase in its utilization in FY 1965. Several important analytical-radiochemical procedures were developed by this Section during FY 1963-1964. These new procedures have enabled other Section Investigators to expand their program objectives, thus substantially increasing the expected results of all the programs presently supported by this Section.

16. EXPECTED RESULTS IN FY 1966

The program outlined for 1964 will carry over, in the main, to FY 1966 when results similar to those outlined for FY 1965 are anticipated. Anticipated results here will depend on the various sections which this Section supports.

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 - 1964

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: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	3	3	4
(b) Other Tech.	½	½	¾
Total	3½	3½	4 ¾

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 38,100	$ 41,800	$ 56,100
(b) Materials & Services	8,600	7,600	10,500
(c) Indirect Expenses*	(3%) 30,500	(3%) 33,300	(3%) 34,000
Total	$ 77,200	$ 82,700	$ 100,600

* 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 1964

1. Quantitative relationships between fallout radioiodine on native vegetation and in the thyroids of herbivores. Program and Abstracts of Papers, Symposium on the Biology of Radioiodine. Hanford Laboratories, Richland, Washington: 19.

2. Increased environmental radiation in Southern Nevada, October-December, 1961. University of California, Los Angeles, U.S. Atomic Energy Commission Report UCLA 518, 45 pp., (with William E. Martin).

3. Food-chain relationships of iodine-131 following two nuclear tests in Nevada. Preliminary Report, Project Sedan, U.S. Atomic Energy Commission PNE-236P. 70 pp., (with William E. Martin).

4. Quantitative relationships between fallout radioiodine on native vegetation and in the thyroids of herbivores. Health Physics 9: 1241-1246.

5. Uptake of radioactive material from a nuclear reactor by small mammals at the Nevada Test Site. Health Physics 10: 65-68, (with Kermit H. Larson et al).

12. SCOPE OF THE PROJECT

Work will continue in problems related to the operation of nuclear reactors and the disposal of radioactive wastes and to local fallout from engineering experiments as they occur.

Of major concern is the accumulation and cycling of radioactive substances in consumer populations and the attendant doses to which individuals of these populations are exposed. Studies of cycling will be augmented by the use of mathematical models which define the time-specific relationships of radioisotopes in various compartments of the environment. In such studies probabalistic formulations are more effective than deterministic; hence the stochastic aspects of food-chain transfers of radioisotopes will be given primary consideration. The transfer of I^{131} to milk from contaminated range forage will be given particular attention.

Studies of the dose experiences of native mammals resulting from internal emitters and exposure to external emitters in contaminated environments were begun with the Sedan test in 1962. These studies will be expanded to include data from the test series of 1955 and 1957, with particular emphasis on I-131 and radiostrontium.

A collaborative project with the Puerto Rico Nuclear Institute was begun in the Luquillo Experimental Forest near San Juan. In early 1963, the Institute initiated a study of the effects of gamma irradiation on a section of tropical forest. A number of subsidiary projects have been developed in which the unifying theme is a consideration of the influences of ionizing radiation on the energy metabolism of the forest.

The most numerous vertebrates in the forest are lizards and frogs. Determining the densities and age-distributions of populations of these animals, and how such attributes may be modified by exposure to gamma irradiation, is an

(2) Radioiodine on native vegetation and in the thyroids of jack rabbits after the Sedan test (Joint project with Dr. William E. Martin).
Following the Sedan test of July 6, 1962, in Nevada, the applicability of mathematical models to food-chain transfers of iodine-131 in natural environments was examined. The amounts of radioiodine measured in the thyroids of jack rabbits collected at 5-day intervals between July 5, and August 5 were compared to those predicted by models on the basis of estimated levels of radioiodine on vegetation as of July 6. Four areas, from 20 to 110 miles from ground zero, were studied between 5 and 30 days after the test. The basic model was deterministic but a probabilistic model predicated on the same assumptions was also developed and tested. The performance of the models was good enough to encourage further work of this nature. We considered it more likely that disparities between observations and predictions were due to errors in estimating input variables than to a flaw in the design of the models. Accurate estimates of daily ingestion of radioiodine, and absorption and uptake by the thyroid were the most difficult to obtain.

Analyses of vegetation samples suggested that the distribution of radioiodine on vegetation after the test was lognormal, not normal. When distributions of radioiodine on vegetation were defined as lognormal and frequency distributions of thyroid radioiodine in large synthetic populations were generated by the computer, these distributions were also log-normal. Whether such distributions reflect the situation in nature depends on the validity of the assumptions built into the model. We believe that the model assumptions were qualitatively sound, and that lognormal distributions of radioisotope concentrations in organs are probably characteristic of populations consuming vegetation contaminated by local fallout. This conclusion was supported both by measurements of radioiodine on vegetation at various times between July 11 and August 5, and by analyses of observed distributions of radioiodine in the thyroids of herbivores consuming this vegetation.

The assumption of the Federal Radiation Council that the "majority of individuals do not vary from the average by a factor greater than three" appears reasonable, both on the basis of actual observations and analyses of synthetic distributions of 1000 individuals.

If our measurements of radioiodine on sagebrush (Artemisia tridentata) in the vicinity of Currant, Nevada (110 miles from ground zero), were even close to levels of radioiodine on cattle forage (e.g., no more than 10 times higher), it is an unavoidable conclusion that had milk been produced in this area during July of 1962, it would have contained radioiodine (2000-3000 $\mu\mu$c/1), far in excess of the limit of Range II recommended by the Federal Radiation Council (100 $\mu\mu$/1). Levels would have been higher in areas closer to ground zero. Fortunately, there is little dairying in these parts of Nevada.

(3) Chronic irradiation of lizards and frogs in a Puerto Rican rain forest.
Following a 2-week preliminary study in July 1963, plans were made for a one-year background survey of populations of lizards and tree frogs. As a result the number of species to be studied intensively was restricted to three: the tree frog Eleutherodactylus portoricensis and the lizards Anolis gundlachi and A. evermanni. In November of 1963 a technician from UCLA moved to San Juan and he has been working in the forest since that time. Two adjacent circular areas 60 m in diameter are currently under investigation. The size and comp-

important feature of the investigation of the metabolism of the community as a whole.

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
Hanford Atomic Products Operation, Richland, Washington
National Reactor Testing Station, Arco, Idaho

14. TECHNICAL PROGRESS IN FY 1964

(1) The effects of a deeply buried nuclear device on lizard populations

Following the Sedan test of July 6, 1962, adult Cnemidophorus tigris, Crotaphytus wislizeni, and Uta stansburiana were exterminated to a distance of 4000 feet from ground zero. Very few adult lizards of any species were observed during August and October of 1962 within 6000 feet of ground zero; but the occurrence of adults between 4500 and 5000 feet during June of 1963 indicated that the post-test observations in 1962 may have reflected seasonal inactivity. Sampling in June of 1963 indicated that the apparent absence of adults within 4000 feet in 1962 was a fact. The immediate mortality in this area is attributed to the gross physical effects of the detonation--dirt fall and blast, which destroyed all of the vegetation out to 2000 feet and caused partial damage to 5000 feet from ground zero.

In June of 1963, between 4500 and 5000 feet from ground zero, Uta was apparently more abundant than Cnemidophorus. It is suggested that Cnemidophorus was more sensitive than Uta to the deleterious influences involved in this study. No changes attributable to the test were detected at 8500-9000 feet.

Eggs of the three species hatched following the test in areas where adults did not survive. Young Uta stansburiana were numerous in August in areas 2600 to 9000 feet from ground zero. By October, the young lizards at 2600 feet were all dead and mortality at 3800 feet was extremely heavy. At 9000 feet the apparent density of young Uta was only slightly reduced as compared to that in August. The delayed mortality is attributed to destruction of habitat. Neither the depletion of food resources nor the residual gamma radiation is considered sufficient to cause death. Absorbed tissue doses, as registered by 3 micro-dosimeters implanted in lizards before the test and recovered on July 28, were similar to doses registered 2" underground and were less than 15% of free-air doses registered in the same area.

osition of populations of the above three species are being determined by repeated sampling and marking. Anolis gundlachi is a large arboreal lizard found at intermediate heights in the forest. The density of this species may approach 1000 per acre! A. evermanni occurs higher in the trees, and may move about in the canopy itself. During rains these lizards move lower in the forest, and it is during these periods that our sampling is most successful. All captured lizards are marked and measured, and their location in the study area recorded. Also recorded are the height at which each animal is taken and the diameter of the tree on which it is captured.

15. EXPECTED RESULTS IN FY 1965

Research on food-chain transfers of radioisotopes will be continued. The approach may be theoretical, and predicated on mathematical modelling; or the efficacy of such models may be tested in real situations should such opportunities arise. The dose-rates and lifetime doses in small mammals calculated from data acquired during earlier test series will become available with particular emphasis on the relationship between dose due to internal emitters and that absorbed from external gamma emitters. Information will become available as to the potential environmental influences of power reactors of the types proposed for several localities in Southern California.

Results of the background studiesin Puerto Rico will become available. Following a full year of investigation, we shall know the seasonal cycles of breeding and other activity exhibited by the lizards and tree frogs, if such cycles do indeed occur. In January 1964, irradiation of one of the 60 meter circles will begin. During December of 1964, glass microdosimeters will be implanted in as many individuals of the three species being studied as possible. These dosimeters will provide an index to the dose experience of the animals. Following about three months of irradiation the experimental and control areas will again be investigated.

An analysis of nuclear volumes of two species of lizards and two species of tree frogs is planned as a collaborative study with Dr. Koo of the Puerto Rico Nuclear Institute. Another collaborative study with Dr. J. Szepsenwol of the San Juan Medical School has been arranged. Dr. Szepsenwol will examine the chromosomes of 6 species (using material from the testes) both before and after the irradiation.

16. EXPECTED RESULTS IN FY 1966

Preliminary results of studies of the possible influences of wastes from power reactors located along the Southern California coast will be available. Results from tests of Kiwi reactors at the Nevada Test Site will be developed, if such tests do occur.

The metabolic rate of individuals of the important species of frogs and lizards in the Puerto Rican forest will have been worked out and these data incorporated into Odum's study of forest metabolism. Age differences in metabolism will have been evaluated, and these differences used in conjunction with information on the age-distribution of populations to estimate the metabolism of entire consumer populations.

The data developed by Szepsenwol on chromosome morphology before and after the irradiation will be correlated with dosimetry data acquired from the irradiated animals.

Experiments on the response of several of the forest lizards to acute gamma irradiation will have been completed, and preliminary data will be available. The response of forest-dwelling species, exposed to little direct insolation, will be compared with that of desert species which are exposed to high levels of solar radiation.

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 - 1964

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: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	4½	4½	4½
(b) Other Tech.	½	½	½
Total	5	5	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 43,900	$ 45,100	$ 46,200
(b) Materials & Services	9,100	5,100	6,600
(c) Indirect Expenses*	(4%) 40,600	(3%) 33,300	(3%) 34,000
Total	$ 93,600	$ 83,500	$ 86,800

* 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 1964

1. Notes on the deposition of fallout in relation to topography and local meteorological conditions. UCLA Rpt. 517. 1963

2. Loss of I-131 from fallout-contaminated vegetation. Health Physics 9: 1141-1148. 1963

3. Increased environmental radiation in southern Nevada from October to December 1961. UCLA Rpt. 518. 1963

4. Food-chain relationships of I-131 following two nuclear tests in Nevada. PNE-236. 1963

5. Losses of Sr-90, Sr-89, and I-131 from fallout-contaminated plants. Radiation Botany. (In press).

12. SCOPE OF THE PROJECT

The purpose of this project is to investigate: (1) the close-in effects of nuclear detonations on vegetation and environment, (2) the role of plants in the trophic-dynamics (food-chain relationships) of radionuclides released to the environment by nuclear detonations or reactors, and (3) the quantitative relationships between radionuclide concentrations on fallout-contaminated plants and in the tissues of herbivores. Such studies are needed to aid in estimating the biological consequences of testing and using nuclear devices for peaceful or for military purposes.

(1) Close-in Effects of Nuclear Detonations on Vegetation

Nuclear war or the widespread use of nuclear explosives for excavation could destroy or damage the vegetation and alter the productive potentials of relatively large areas. While the immediate effects of a nuclear detonation on vegetation and environment may be quite spectacular, the long-term effects may be similar to those associated with conventional methods of excavation and other modes of disturbance such as fires, floods, and the like.

Previous studies in this research area have been quite limited. Our studies to date have dealt only with the immediate effects of a single underground detonation (Project Sedan) on the soil and vegetation within a radius of about 2.5 mi. from GZ. These include both preshot and postshot estimates of basic vegetation parameters and soil properties. Long-term studies are anticipated, and it may become desirable to expand this program to include similar studies in connection with future events in the Plowshare Program.

(2) Role of Plants in the Trophic-Dynamics of Radionuclides

Radionuclides produced by nuclear detonations or by nuclear reactors may be released to the atmosphere and subsequently desposited on soils and plants in terrestrial ecosystems. The materials deposited directly on plants may be ingested by herbivores and thus gain immediate entry into

food-chains leading to man. The materials deposited on soil are potentially available for redistribution, but their entry into food-chains leading to man is generally delayed.

Previous studies have shown that plant foliage may intercept as much as 10% of the fallout deposited on a unit area of crop plants. While 50% of the external activity may be removed by wind and rain, contamination tends to persist until defoliation occurs. Other studies have indicated that most of the Sr-90, for example, in the bones of rabbits living in fallout contaminated areas may have been acquired during relatively short periods of external plant contamination.

These results indicate that the source-to-plant-to-herbivore route of radionuclide entry into terrestrial food-chains may be more significant than the source-to-soil-to-plant-to-herbivore route of entry which has received much more attention. To investigate this possibility studies are now being made or planned to: (1) estimate the fraction of fallout or reactor effluent intercepted by different kinds of vegetation under different conditions of contamination, (2) to estimate the concentration of radionuclides on vegetation in relation to distance from the source and to time after contamination, (3) to estimate the rates at which radionuclides are lost from vegetation contaminated by fallout or reactor effluents, and (4) to determine how these rates may be influenced by wind, rain, and other environmental or physiological factors.

13. RELATIONSHIP TO OTHER PROJECTS

(1) Close-in Effects of Nuclear Detonations on Vegetation

This project concerns the immediate and the long-term effects of Project Sedan on vegetation. Dr. J. C. Beatley, of this Laboratory, is studying the effects of Project Sedan on vegetation in two permanent plots located approximately 5,000 ft. and 10,000 ft. from ground zero. Dr. F. B. Turner of this Laboratory, has made a similar study of lizard populations; and Dr. Dorald Allred, of Bringham Young University, is making a study of small mammal populations.

Studies of vegetation in other target areas at the Nevada Test Site, areas denuded or damaged by nuclear tests during the 1950's, have been made by Drs. L. M. Shields and P. V. Wells of Highlands University. Similar studies have been made at Eniwetok Atoll by Dr. R. F. Palumbo of the University of Washington.

(2) Role of Plants in the Trophic-Dynamics of Radionuclides

Most of the research projects in the Environmental Radiation Division of this Laboratory are directly or indirectly concerned with some aspect of the biological cycling of radionuclides. Studies made in cooperation with Dr. F. B. Turner have contributed to the development of mathematical models to account for the build-up and decline of I-131 in the thyroids of rabbits living in areas contaminated by fallout. Similar studies should provide information which will supplement the findings of Drs. H. A. Hawthorne and E. M. Romney.

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 is directed by Dr. S. I. Auerbach at the Oak Ridge National Laboratory. The Oak Ridge program is described in TID-16890.

14. TECHNICAL PROGRESS IN FY 1964

(1) Close-in Effects of Nuclear Detonations

Studies previously initiated in the vicinity of Sedan ground zero have been continued. The invasion of radioactive throwout deposits by Salsola kali and other annuals and the recovery by crown sprouting of blast-damaged shrubs (2000 to 5000 ft. from ground zero) have been observed and documented by photography and the collection of population data. Similar observations and measurements have also been made in the base-surge dust zone (ca. 5000 to 10,000 ft. from ground zero), but it is not yet clear whether the damage observed in the dust zone should be attributed to dust deposition or to ionizing radiation or to both.

(2) Role of Plants in the Trophic-Dynamics of Radionuclides

Plant samples collected on the 5th, 15th, 30th, and 60th days after the detonation from 20 locations in the Sedan fallout field were analyzed to determine their Sr^{89} and Sr^{90} content ($\mu\mu c/g$) at the time of collection. From 5 to 30 days after the detonation the average effective half-lives of Sr^{89} and Sr^{90} on fallout-contaminated plants were 17.8 days and 27.8 days respectively. From 30 to 60 days after the detonation, the average effective half-life of Sr^{89} on plants was 37.6 days and that of Sr^{90} was 152 days. Losses in excess of radioactive decay were attributed to the mechanical removal of fallout particles, primarily by wind action.

Studies were also made to determine the size and number of radioactive particles per unit area of leaf surface in relation to time after fallout and location in the fallout field. Results to date seem to indicate that wind action can result in either an increase or a decrease in the total number of fallout particles present on foliage at a given time after fallout, but the number of particles per unit area of leaf surface is not correlated with gross gamma activity or radionuclide content.

(3) Food-chain Relationships of Sr^{89} and Sr^{90}

The bone ash of rabbits collected from the Sedan fallout field at 5-day intervals for the first 30 days after fallout and again 60 days after the detonation were analyzed to determine the concentrations ($\mu\mu c/g$ bone ash) of Sr^{89} and Sr^{90} at the time of collection. Preliminary analyses of these data indicated that maximum or near maximum levels of Sr^{89} and Sr^{90} in rabbit bone ash were attained during the first 30 days after the detonation. From 30 to 60 days after the detonation, there were no significant increases.

It has been demonstrated that the average rate of increase of Sr^{89} and Sr^{90} in rabbit bone from 5 to 30 days after fallout was approximately exponential. Further studies are being made to develop mathematical models to express the quantitative-time relationship between radiostrontium levels on fallout-contaminated plants and in the bone ash of herbivores.

15. EXPECTED RESULTS IN FY 1965

(1) Close-in Effects of Nuclear Detonation

Studies will be continued to describe or measure: (a) the development of vegetation on radioactive throwout deposits, (b) the recovery of blast-damaged plants, (c) the growth, developmental, and reproductive behavior of shrubs exposed to base-surge dust deposits and cumulative gamma doses in excess of 4000 r, and (d) the radionuclide content of soils and plants in relation to distance from ground zero. Publication of an interim report (PNE-228F) is anticipated.

(2) Role of Plants in the Trophic-Dynamics of Radionuclides

It is anticipated that the studies related to Project Sedan will be completed and that the significant results will be reported during FY 1965.

(3) Food-chain Relationships of Sr^{89} and Sr^{90}

It is anticipated that the studies related to Project Sedan will be completed and the significant results will be reported during FY 1965.

16. EXPECTED RESULTS IN FY 1966

(1) Close-in Effects of Nuclear Detonations

Studies initiated from 1962 to 1965 will be continued as long as feasible and fruitful.

(2) Role of Plants in the Trophic-Dynamics of Radionuclides, and

(3) Food-chain Relationships of Radionuclides

Until studies currently in progress have been completed, it will be difficult to determine the exact nature of further studies. Preliminary results indicate that more work should be done on the role of plants in the interception, retention, and food-chain transfer of radionuclides contained in fallout. Studies are also needed to determine rates of radionuclide assimilation and biological elimination by herbivores. Both laboratory and field studies are needed.

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 and Radiological
Characteristics of Fallout

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

4. Date Prepared:
April - 1964

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

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
K. H. Larson

8. Project Term:
From: 1957 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2	2	3
(b) Other Tech.	-	-	-
Total	2	2	3

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 26,800	$ 28,600	$ 36,300
(b) Materials & Services	1,400	1,400	2,000
(c) Indirect Expenses*	(2%) 20,300	(2%) 22,200	(2%) 22,700
Total	$ 48,500	$ 52,200	$ 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 1964

Operation Sunbeam - Shot Small Boy. "Fallout collection and gross sample analysis". Project Officers Report - Project 2.9-POR-2215. Part I by P. D. LaRiviere, J. D. Sartor, W. B. Lane; U.S. Naval Radiological Defense Laboratory, San Francisco. Part II by K. H. Larson, Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles. In Press.

Reconnaissance Group - Site Selection - Project Rufus. "Project Rufus - Site Selection - Reconnaissance Report, Alaska" by Rudolph A. Black, Garth B. Harlan, K. H. Larson, Robert E. Lyle, Wm. S. Twenhofel, and Luke J. Vortman. Published by USAEC - Nevada Operations Office, June 1963, 150 pp. classified secret. (This report is the result of a combination of arrangements and joint agreements between this Laboratory, Hazelton Nuclear Science Corp. Palo Alto, California and the Nevada Operations Office.)

Damage to Livestock from radioactive fallout in the event of nuclear war. A report by the sub-committee on livestock damage of the Advisory Committee on Civil Defense, National Academy of Sciences - Nation Research Council Dr. John H. Rust, Chairman. NAS-NRC publications 1078 (1963).

The behavior of radioactive fallout in soils and plants. A review prepared for the Committee on Effects of Atomic Radiation on Agriculture and Food Supplies, National Academy of Sciences - National Research Council by Frere, M. H., R. G. Menzel, K. H. Larson, Roy Overstreet and R. F. Reitemeier. NAS-NRC publications 1092 (1963).

Contamination of plant foliage with radioactive fallout. Romney, E. M., R. G. Lindberg, H. A. Hawthorne, B. G. Bystrom, K. H. Larson. Ecology 44:2 343-349 (Spring 1963). Also as USAEC report TID 15870. 30 pp.

Root transfer of fission products from contaminated soil. Romney, E. M., H. Nishita, J. H. Olafson, K. H. Larson. Soil Sci. Proc. 27(4): 383-385 (July-August, 1963).

Uptake of radioactive material from a nuclear reactor by small mammals at the Nevada Test Site. Turner, F. B., B. W. Kowalewsky, R. H. Rowland, K. H. Larson. Health Physics 10(2): 65-68 (Feb. 1964). Also as USAEC report TID-18864.

12. SCOPE OF THE PROJECT

During the period FY64-FY66, this project is and will be concerned with comprehensive review, evaluation, integration and publication of the work of this Laboratory during the years from 1947-1964, which is related to the distribution, characterization and biological significance of radiation fission products released from nuclear detonations in continental United States. This endeavour is of value since the data to be evaluated comprises the earliest, 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 organi-

zations (e.g. Stanford Research Institute, AEC, DBM, 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 because of the partial nuclear test ban, predictions must be based on extrapalations 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. Attempts to develop new scaling factors which are based on such data will amplify the original uncertainties.

It is our intention to evaluate, interpret and report significant data which we have accumulated and to realistically evaluate 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 in preparing for the possibility of nuclear warfare.

In view of these considerations and the interest in the distribution in the biosphere of specific radionuclides, such as radioiodine, at greater distances from ground zero than customarily considered before 1962, we propose the following program:

1. Evaluate and report unpublished work of this Laboratory related to distribution of close-in radioactive fallout debris in the biosphere of the environs of NTS from 10 to 250 miles from ground zero;

2. Evaluate and report data available from representative fallout patterns on the characteristics of fallout e.g. solubility of radioactivity, concentrations of radionuclides and the ratios of Sr/Zr, Cs/Zr, Ru/Zr with respect to weapon yield, time of fallout, particle size, and type of weapon mounting;

3. Review and evalutate the methodology of collection and analysis used to obtain these data;

4. Establish an estimate of the combined effect of several environmental factors on the rate of immoblilization of fission products and plutonium in fallout patterns, utilizing as a basis the observations made in the Trinity Area, New Mexico and certain areas in Nevada.

This Laboratory is one of the few institutions that has a reasonably complete library of fallout samples from continental detonations. It will be possible, as the evaluation and reporting of previous work progresses, to analyze selected samples using the more refined and current available techniques for additional radionuclides such as I-129 (half life: 1.6×10^7 yrs) or to recheck the reliability of previously used processing methods and procedures. It is felt this effort will make a significant and unique

contribution to specific problems of the overall task of fallout assessment which other organizations have recently set up to accomplish from a primarily theoretical basis.

13. RELATIONSHIP TO OTHER PROJECTS

Plowshare Group, Lawrence Radiation Laboratory
Off-site Radiological Safety Group - USPHS/USAEC-NVOO
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 PROGESS IN FY 1964

(a) The final Project Officer's report for the NMRB/UCLA portion of the fallout study, from 18 to 200 miles from ground zero, was completed and submitted to USNRDL, as Part II of the POR-2215, "Fallout collection and gross sample analysis (u)"; Project 2.9, USNRDL/UCLA, Operations Sunbeam - Shot Small Boy (classified secret, RD). It has been approved and submitted for publication by DASA-DOD. Some of the results obtained are as follows:

1. The gamma decay curve developed from data obtained from gamma-intensity-time recorders (GITR) and decay measurements on collected fallout samples to H + 40 hrs. indicated that the decay factor $t^{-1.2}$ (range of $t^{-0.97}$ to $t^{-1.4}$) was the best factor for this particular fallout debris.

2. There were larger radioactive particles found in fallout samples than in the original soil particles found at ground zero.

3. The rate of travel of the "cloud" over the landscape was variable. Also, there was indication that the portion of the cloud above 16,000 ft. MSL sheared to the east-southeast resulting in small amounts of radiation being measured by the GITR's and time-of-arrival detectors (> 2 mr/hr) but no fallout material was deposited at these locations.

4. No relation was apparent between the radioactivity per unit area and mass per unit area within the fallout pattern from 18 to 200 miles from ground zero.

5. A maximum of seventy per cent of the activity was associated with the greater than 44 micron size fraction at 18 miles from ground zero; at 200 miles from ground zero, 40 per cent of the activity was associated

with the same size fraction.

6. These data suggest that activity per sq. ft. varied inversely with the time of fallout arrival, i.e. stations near the hot-line of the pattern from this detonation tended to have earlier fallout arrival than stations toward the periphery of the pattern. This phenomenon was observed particularly on the 18 and 27 mile-distant arcs from GZ.

7. Adequate samples were furnished USNRDL Project 2.10 for radionuclide determinations, as per agreement.

(b) Work is progressing on the final reports on fallout studies done on Shot Sedan, from 7 to 70 miles from ground zero (CETO Project 62.86) and the collection of fallout from 2500 feet to 25,000 feet from ground zero on Shot Danny Boy (UCLA Project 2.4). Special effort has been made to determine the reliability of these data obtained by the particular Methods Procedures and field equipment used in this study. This evaluation should allow recommendations as to studies and their methods which may provide more reliable data on the phenomenology of fallout and its characterization and distribution in any future cratering experiments.

(c) A cooperative effort between this Laboratory and the Hazelton-Nuclear Science Corp., Palo Alto, California was continued on a special classified study of site selection. The participation of the senior investigator was requested by NVOO through DBM. The study was completed after six weeks of a reconnaissance trip surveying and evaluating potentially acceptable sites as a part of the classified project "Rufus". The classified report, cited above in Item 11, presented the findings of the Rufus reconnaissance team of six people, representing several scientific disciplines from four organizations.

15. EXPECTED RESULTS IN FY 1965

(a) Complete the final Project Officer's reports on fallout studies done by this Laboratory for the following events:

Shot Sedan (1962) - Report No. PNE-225
Shot Danny Boy (1962) - Report No. WT-1818
Plumbob Test Series (1957) - Report No. WT-1488

(b) Review and report available data and apparent relationships concerning re-distribution and immobilization of radioactive fallout debris from above-surface NTS detonations in the environs of NTS. This review will be based on studies and observations made by field groups before 1961 and will include:

(1) an evaluation of measurements made during the 1952-1961 persistance studies of radioactive contamination in selected areas in Nevada and Utah;

(2) an analysis of monthly measurements of debris collected by stainless steel pots during 1959, 1960, and 1961;

(3) an evaluation of daily fallout tray collections and air sampling at selected locations for two week periods in 1957 and 1958;

(4) an evaluation of the relation between activity per unit area to mass collected per unit area, particle size spectrum, solubility of activity and biological availability.

16. EXPECTED RESULTS IN FY 1966

(a) Review and summarize available information and data in Technical Memoranda, Special AEC reports and unpublished work on fallout from the Trinity Shot, New Mexico and its fate in the biosphere. The several memoranda and reports from the Los Alamos Scientific Laboratory issued before 1949 when integrated with the information obtained by this Laboratory from 1947-1953 would present a history of plutonium and fission products in the biosphere. This could be extended to cover a span of 20 years by making another collection and analysis of soil, vegetation and indigenous rats and rabbits. The five study areas that were established beyond the crater fence along the fallout pattern are still relatively undisturbed by man, (personal observation March 1964).

(b) Evaluate and present a review of UCLA studies on surface and aerial radiation intensity measurements made in fallout patterns from above-surface nuclear detonations in the Teapot Series (1955), Plumbob Series (1957), Hardtack II Series (1958), and the Sedan and Small Boy events (1962). This would include:

(1) a comparison of radiation measurements made with the survey meters available to the several research projects and support groups;

(2) a comparison of radiation intensity and dose measurements made by UCLA film packs, survey meters, gamma intensity recorders and Arms I and Arms II aerial radiation measurements.

(3) a comparison of measurements with respect to time after fallout and location within the fallout pattern.

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 Dosimetry

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

4. Date Prepared:
April - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
George V. Taplin

8. Project Term:
From: 1949 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2 1/4	1 1/4	1 1/4
(b) Other Tech.			
Total	2 1/4	1 1/4	1 1/4

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 22,000	$ 16,500	$ 16,500
(b) Materials & Services	2,800	2,400	2,400
(c) Indirect Expenses*	(2%) 20,300	(1%) 11,100	(1%) 11,300
Total	$ 45,100	$ 30,000	$ 30,200

* 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 1964

1. Nishihara, N., Taplin, G.V., Carpenter, C.: Immunogenicity of gamma irradiated mycobacterium tuberculosis H37Rv(GIV) mice. Amer. Rev. Resp. Dis. (in press).

2. Taplin, G.V., Malin, K.M.: Tetrazolium-blue gelatine dosimeter systems. USAEC progress report UCLA-520, 70-71, 1963.

12. SCOPE OF THE PROGRAM

Many problems in radiation dosimetry remain incompletely solved by physical methods, such as measurement of mixed neutron-gamma exposures from nuclear reactor excursions, tissue penetrability of low energy X-rays, beta and alpha particles and accurate measurement of high intensity radiation sources in the mega rad range. The major objective of this project is to develop tissue equivalent chemical methods for solving dosimetric problems not possible or practical by other means. In this respect the tetrazolium blue dye in polyvinyl alcohol plastic (TB-PVA) system has many unique properties and capabilities. With TB-PVA film dosimeters one can perform high level dosimetry of X, γ, and beta radiations and make actual measurements of depth dose distribution in tissue equivalent density material. Additional objectives are to use our radiation facilities and dosimetric techniques in collaborative radiobiological studies with other projects in this laboratory and with various departments of the School of Medicine to advance the understanding of biological effects of radiation at the chemical level and to develop new beneficial uses of radiation and dosimetry.

13. RELATED PROJECTS

Similar investigations are being conducted at the Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee; Los Alamos Scientific Laboratory, Los Alamos, New Mexico; Brookhaven National Laboratory, Upton, New York; Stanford University, Palo Alto, California.

This program is conducted in collaboration with other divisions within this laboratory, Dr. Benedict Cassen (biophysics) Dr. Lawrence Myers (radiobiology) and as a service function to the Department of Radiology, Division of Radiation Therapeutics (Dr. Justin Stein).

14. TECHNICAL PROGRESS IN FY 1964

A tetrazolium blue-gelatin dosimeter system was developed during the past year. It has several advantages over the previously described tetrazolium blue dye-polyvinyl alcohol plastic dosimeters in that it may be prepared in various sizes, shapes and forms and may be loaded into aluminum tubes and read end-on. The long tubes provide amplification of the radiation response and thereby permit measurement in lower dose ranges. The higher water content of the gelating system gives it greater radiation sensitivity and its density is more nearly tissue equivalent. Furthermore, gelatine systems are readily prepared from stock solutions in the laboratory and may be used immediately for a variety of experimental purposes. The radiation sensitivity may be adjusted over wide ranges by varying the concentration of the dye. The G value for dye reduction

increased with dye concentrations in similar fashion to the PVA-TB system. This observation indicates that the reverse of the dilution effect prevails in both of these systems. Cross calibration experiments with our two Co^{60} facilities indicate that the tetrazolium blue-gelatin and PVA systems are equally reliable indicators of gamma radiation exposure in the dose range of 1-6 x 10^5 r and accurately register exposure at dose rates between 1200 and 1500 r/min.

The Tetrazolium Blue-Polyvinyl Alcohol Film Dosimeter - A comprehensive paper on this subject is in preparation. It covers the principles of the system, describes the methods of preparing the films, their radiation characteristics, means for altering their radiation response, the effects of additives including Li^6 and B^{10} for thermal neutron measurement and finally presents several useful applications.

Collaborative work in the radiation exposure of lyophilized bacterial vaccines has been continued with the Department of Infectious Diseases at UCLA. TB-PVA dosimeters are used to monitor these exposures with the objective of destroying the reproductivity capacity of the organisms without reducing their antigenicity.

15. EXPECTED RESULTS IN FY 1965

Work with the tetrazolium blue dye-gelatine and tetrazolium blue-polyvinyl alcohol systems will be continued to devise more sensitive tissue equivalent density dosimeters for application in the biologically interesting dose range (100-5000 r). Emphasis will be placed on learning more about the mechanisms involved in these tissue equivalent systems, particularly in respect to reaction kinetics as influenced by temperature variations including the frozen state. Both systems will be investigated for applicability as high range dosimeters in food and drug sterilization programs. Collaborative dosimetric studies will also be continued with the Department of Radiology in devising improved radiation therapeutic techniques for the treatment of deep seated tumors using the Co^{60} teletherapy facility and the 6 mev linear accelerator.

16. EXPECTED RESULTS IN FY 1966

No major change in scope or magnitude of the research in this section is anticipated. Studies on solid state and semi-solid state tetrazolium blue-polyvinyl alcohol plastic and TB dye-gelatine dosimeters will be continued. Emphasis will be placed on developing specific applications in radiobiology (thin film dosimeters)and in the therapy of deep seated tumors with high energy x-ray beams (6 mev linear accelerator). A search for alternate oxidation reduction dyes will be continued to find ones having equal stability but greater radiation sensitivity than those in the tetrazolium series. Further work will be done to modify our current read out devices (photoelectric colorimeter) so that cylindrical plastic dosimeters may be read end-on to amplify the radiation induced changes in the solid state systems.

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.:	4. Date Prepared:
06-06-02	April - 1964
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: 1955 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	3 1/4	3 1/4	3 1/4
(b) Other Tech.			
Total	3 1/4	3 1/4	3 1/4

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 35,200	$ 36,300	$ 36,300
(b) Materials & Services	10,100	4,000	4,000
(c) Indirect Expenses*	(3%) 30,500	(2%) 22,200	(2%) 22,700
Total	$ 75,800	$ 62,500	$ 63,000

* 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 1964

Theory of the Performance Characteristics of Radioisotope Distribution Imaging Systems, B. Cassen. J. Nuclear Medicine, Feb., 1964

12. SCOPE OF THE PROGRAM

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 two major efforts in progress as well as other lesser developments:

The first, which is well under way, is that of exploring new possibilities of appreciably increasing gamma quantum utilization efficiency in radioisotope scanning. As pointed out in the above publication, this method which consists in greatly increasing the solid angle of radiation detected per resolution element, provides a possibility of obtaining appreciable performance improvement.

The second major effort consists in developing new procedures for the separation of cell suspensions into groups having different physical characteristics. As a first phase of this development, methods were perfected for using a multi-channel analyzer with a Coulter counter for very rapid determination on a small sample of a complete spectrum of size distribution of a cell suspension. The major phase of this development is the perfection of an electrophoretic separator for the actual gross separation of living cells into groups differing in zeta potential. The determination of size spectra after electrophoretic fractionation shows that with both erythrocytes and separated lymphocytes there is some correlation of size and zeta potential (see 14 below).

Some pilot studies have been made to determine the advantages and feasibility of producting highly localized radiation lesions with a 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. The experimental results appear to be very promising, (see 14 below).

13. RELATIONSHIP TO OTHER PROJECTS

a. 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, Mass.
Drs. Bender and Blau, Roswell Memorial Institute, Buffalo, N. Y.
Dr. H. Anger, Donner Lab. University of California, Berkeley, California
Dr. Beck, Argonne Cancer Hospital, Chicago, Ill.
Dr. Harris, Oak Ridge National Laboratory.

b. Physical Cell Measurements and Separation

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

c. Scintillation Hemodynamics

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

14. TECHNICAL PROGRESS IN FY 1964

a. The possibility of increasing radioisotope gamma scanning utility in clinical applications was explored by building and completing a high quantum utilization scanner. The detecting unit is a spherical 2,200 hole collimator, about 14" in diameter and a semiangle to the focus of about 60 degrees. This collimator and shielding is driven hydraulically under the patient. The system as finally tested functions as it was designed to. It has been moved and installed in the clinical diagnostic neurosurgery space at the UCLA School of Medicine where a clinical patient test program will be initiated.

b. On account of the recent tremendous resurgence of interest in medical radioisotope scanning, the original scanner (Report UCLA 130, 1951) which was disassembled a few years ago, has been reassembled for its historical interest.

c. Physical Cell Measurements and Separations

In studying the effect of radiation on the size distribution of separated lymphocytes a procedure was perfected for rapidly determining with high resolution the complete size distribution spectrum of the lymphocytes separated from about 1 cc of blood from an irradiated rabbit. On account of the small volume of blood needed per determination, a single irradiated rabbit can be followed sequentially in time by taking hourly or daily blood samples. Thereby, a tremendous improvement in the statistical quality of the observational data is obtained relative to the use of different animals for each time and dose level. Some of the radiobiological results obtained to date are indicated in the section Medical Physics Problems under A.E.C. Budget Activity 06-06-01.

An electrophoretic cell separator has been built. Many features have been developed to make it a practical, workable device for fractionating blood cell suspensions, and especially separated lymphocytes. It has been demonstrated that with electrophoretic fractionation of erythrocytes or leukocytes or with separated lymphocytes it is possible to determine changes in the size distribution spectrum. This instrument development will enable many new types of measurements on cell suspensions. See Medical Physics Problems under AEC Budget Activity 06-06-01.

d. Theoretical calculations have been made to show that a highly concentrated dose of radiation can be obtained in tissue in vivo by double angle rotation of a penumbraless pencil-like x-ray beam always aimed at and rotating around the region to be dosed. A preliminary test on the UCLA 6 mev. linear accelerator shows that experimental results can approximate the theoretical conclusions. Dose concentrations approximate those that could be obtained from ultra high energy proton or alpha beams. Apparently it would be possible to produce localized radiation lesions in the brain both experimentally with animals and in brain tumor radiation therapy.

15. EXPECTED RESULTS IN FY 1965

It is expected that the clinical testing program of the 2,200 hole collimator, high quantum utilization scanner will be well under way and that possible improvements indicated by the results of the program can be incorporated into the equipment. It is expected that improved devices for transferring stereotaxic scanning information to a 3-dimensional rotational radiation lesion production system can be designed and built.

It is expected that procedures of cell size spectra determinations and electrophoretic cell separation procedures can be steadily improved and be made more useful in biological research and hematology.

16. EXPECTED RESULTS IN FY 1966

It is expected that a program of developing methods of transferring stereotaxic scanning information to a 3-dimensional rotational radiation lesion production system will continue. (See 14d above). It is expected that a method can be developed for the production of highly localized radiation lesions in brains of animals and be available for enabling studies of the functions of the central nervous system. It is expected that a system of localized irradiation of brain tumors of patients can become available for therapy. The information for aiming the center of rotation of the x-ray beam at the tumor will be obtained from stereotaxic coordinates obtained from scanning (See 14a 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:
Chemical Toxicity of Rare Earths

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

4. Date Prepared:
April - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	2 ½	2 ½	2 ½
(b) Other Tech.			
Total	2 ½	2 ½	2 ½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 25,300	$ 28,600	$ 29,700
(b) Materials & Services	2,700	2,700	3,400
(c) Indirect Expenses*	(2%) 20,300	(2%) 22,200	(2%) 22,700
Total	$ 48,300	$ 53,500	$ 55,800

* 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 1964

1. Toxicity of Europium Chloride, Haley, T.J., Komesu, N. and Colvin, G., The Pharmacologist 5, 231 (1963).

2. Skin Reaction to Intradermal Injection of Rare Earths, Haley, T.J. and Upham, H. C., Nature 200, 271 (1963).

3. Experimental Usefulness of the Kangaroo Rat, Haley, T.J., Lab. Animal Care, in press.

4. Brain Concentration of Reserpine-H^3 and its Metabolites in the Mouse, Maggiolo, C. and Haley, T.J., Proc. Soc. Exp. Biol. Med. 115, 149-151 (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 nosious 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 1964

Studies of the pharmacodynamics, covering both the intact animal and isolated organ systems, and local toxicological effects on the eye and skin should result in a completion of the general toxicology of praseodymium, neodymium and lutetium. This work is essential for establishment of the toxicological parameters necessary for the establishment of industrial hygienic standards.

15. EXPECTED RESULTS IN FY 1965

Studies on the local and systemic toxicological effects of elements 59 through 71 have indicated the greatest source of damage is local destruction of the skin. Similar studies will be undertaken with other rare elements to determine the importance of such local toxicological manifestations in the overall toxicity of these elements.

16. EXPECTED RESULTS IN FY 1965

Investigation will be made of local and systemic toxicological effects of other rare elements such as ruthenium, rhodium and any 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 in industrial processing.

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.:	4. Date Prepared:
06-09-00	April - 1964
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: Esther Fincher Hays, M. D.	8. Project Term: From: 1955 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	2 ½	2 ¼	2 ¼
(b) Other Tech.	2	2	2
Total	4 ½	4 ¼	4 ¼

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 34,900	$ 33,000	$ 33,000
(b) Materials & Services	6,400	6,400	6,400
(c) Indirect Expenses*	(3%) 30,500	(2%) 22,200	(2%) 22,700
Total	$ 71,800	$ 61,600	$ 62,100

* 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 IN FY 1964

1. Hays, E.F.: The function of the thymus. Observations of lymphoid and hematopoietic tissue in mice after neonatal thymectomy. Rounds of the Teaching Staff, V.A Hospital, Los Angeles, No. 7-10, June 1963.

2. Hays, E.F., White, E.: The development of leukemia in lethally irradiated mice protected with cells from mice of a high incidence leukemia strain. Blood (in press).

3. Hays, E.F.: The effect of deoxyribonucleic acid from normal and leukemic lymphoid tissue on the incidence of leukemia on AKR x C3H F_1 hybrid mice. Nature (in press).

12. SCOPE OF THE PROJECT

This project is concerned with a study of etiological factors in mouse leukemia. Studies are continuing to amplify our initial observations of leukemogenic and tumorgenic properties of deoxyribonucleic acid (DNA) extracted from leukemic cells of mice with spontaneous disease.

A cell free passage of a leukemogenic virus (Gross) is being maintained in our C3HeB and AKR inbred mouse strains. Studies are in progress to (1) demonstrate oncogenic properties of nucleic acid of this virus; (2) determine the histogensis of the viral induced leukemia and (3) perfect an in vitro system for its cultivation.

The thymus is intimately associated with the development of spontaneous and radiation induced mouse lymphocytic leukemia as well as the establishment of the normal lymphoid system in the developing animal. Morphologic studies have been undertaken in neonatally thymectomized mice of high and low incidence leukemia strains to determine if genetic differences in response to this procedure exist. Also we are studying the effect of thymectomy on the development of immunity to small doses of leukemic cells in young adult mice of the same strain (AKR).

13. RELATIONSHIP TO OTHER PROJECTS

Studies of etiological factors in mouse leukemia have been and are being undertaken by many investigators. The following list mentions a few who have made outstanding contributions to the field. Dr. R. Latarjet, Radium Institute, Paris, France; Dr. L. Gross, VA Hospital Bronx, N.Y.; Dr. H. Kaplan, Stanford University Medical School, Stanford, California; Drs. Maloney, Law, Stewart, and Rauscher at the National Cancer Institute, Bethesda, Maryland; Dr. J. Furth Francis Delafield Hospital, New York City; Dr. C. Friend, Sloan-Kettering Institute, New York City; Dr. A. Graffi Berlin, Germany and Dr. J.F.A.P. Miller, Chester Beatty Institute, London, England.

14. TECHNICAL PROGRESS IN FY 1964

Studies have been carried out which demonstrate that DNA extracted from leukemic cells of AKR mice is associated with an increased incidence of leukemia in male offspeing of a hybrid cross of AKR males with C3H females.

16. EXPECTED RESULTS IN FY 1966

The program concerning studies of etiological factors in leukemia will be continued with emphasis on the interrelationships of viral and radiation leukemogenesis. After irradiation or leukemogenic virus injection there is a disappearance or reduction in number of the cortical lymphocytes and a persistance of the epithelial reticular cells of the medulla. We plan to initiate studies to elucidate the role of the epithelial reticular cells of the thymus in the development of leukemia after irradiation or virus infection.

Thymectomy prevents lymphoid leukemia induction by viruses and irradiation and subcutaneous grafts of whole thymus restores the susceptibility to induction of leukemia by these agents. We propose to utilize grafts of thymic reticulam in thymectomized irradiated or virus inoculated hosts and to determine their effect on the development of leukemia in these animals.

No such increase was noted in animals given DNA from normal AKR or C3H lymphoid tissue. The animals were all inoculated within 48 hours of birth.

Incubation of normal thymus cells with leukemic cell DNA in vitro resulted in one lymphoma in 59 animals inoculated with these cells when newborn. No lymphomas were seen in 56 animals receiving thymus cells incubated in DNA from normal lymphoid tissue.

Intracerebral inoculation of leukemia cell DNA however, resulted in 5 leukemias occurring at 9-13 months of age in 29 mice so treated. No leukemias were found in 29 saline inoculated littermate controls.

Studies have been completed which have demonstrated that the previously observed tumor-genic properties of DNA from parotid gland tumors were due to infectious polyoma virus DNA and that DNA extracted from transplanted parotid tumor cells presumably free of virus did not result in tumors after injection into newborn mice. The highly potent oncogenic viral DNA could be extracted from tumors in presence of a high titer of antiviral antibody, but only resulted in tumors when innoculated in animals under 2 weeks of age.

The technic of neonatal thymectomy has been perfected and histological observations of mice of both high and low incidence leukemia strains show a similar pattern of lymphoid atrophy beginning at one week of age. The animals of both strains manifested poor growth and development and died prematurely at 2-3 months of age.

A group of neonatally thymectomized high incidence leukemia strain mice have been isografted with neonatal thymus incubated in normal cell DNA at 10 days of age while their littermates have received allografts of thymus from low leukemia incidence strain mice. These animals are now under observation for the development of leukemia.

15. EXPECTED RESULTS IN FY 1965

The neonatally thymectomized and grafted high incidence leukemia strain mice will be observed for the development of leukemia in the graft and for the occurrence of generalized leukemia. These experiments were designed to show if a reduction of leukemia incidence can occur under these circumstances. C3HeB mice (low incidence leukemia) will be neonatally thymectomized grafter with isogeneic "leukemic" DNA incubated thymus and allogeneic thymus from the high incidence strain to determine if the leukemia incidence in this strain can be increased.

Experiments will be undertaken to study the role of the thymus in tumor immunity.

Studies will continue to attempt to achieve a suitable in vitro method for cultivation of the Gross leukemia virus.

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 - 1964

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: 1958 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	5	6½	6½
(b) Other Tech.	1	1	1
Total	6	7½	7½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 59,900	$ 81,900	$ 83,100
(b) Materials & Services	15,100	9,500	10,500
(c) Indirect Expenses*	(5%) 52,400	(5%) 55,900	(4%) 45,400
Total	$ 127,400	$ 147,300	$ 139,000

* 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 1964

1. Dore, E.K., Taplin, G.V., Johnson, D.E.: Current interpretation of the sodium iodohippurate I^{131} renocystogram. JAMA 185:925-932, 1963.

2. Taplin, G.V., Dore, E.K., Johnson, D.E.: The quantitative radioreno-gram for total and differential renal blood flow. J.Nucl. Med. 4:404-409, 1963 and USAEC Report UCLA-514, June 1963.

3. Taplin, G.V.: The hemodynamics and functions of internal organs (measured externally with radioisotope techniques). Nucleonics 22:58-60, 1964.

4. Taplin, G.V., Johnson, D.E., Dore, E.K., Kaplan, H.S.: Suspensions of radioalbumin aggregates for photoscanning the liver, spleen, lungs and other organs. J. Nucl. Med. (in press) and USAEC Report UCLA-519, November 1963.

5. Dore, E.K., Taplin, G.V., Johnson, D.E.: Relative renal blood flow (clinical and experimental basis of the renogram). Clin. Res. 12:122, 1964.

6. Taplin, G.V., Johnson, D.E., Dore, E.K., Kaplan, H.S.: Diagnostic value of radioisotope procedures in liver and kidney disease. Amer. Coll. Physicians Postgrad. Course on Nucl. Med. and Rad. Biol., Lab. of Nucl. Med. and Rad. Biol., UCLA, Jan. 6-10, 1964. (abstract)

7. Taplin, G.V., Dore, E.K., A.T. Cockett, Johnson, D.E.: Radioisotopes in the diagnosis of renal hypertension, exhibit 1, J. Nucl. Med. May 1964. (abstract).

8. Taplin, G.V., Griswold, M.L., Johnson, D.E., Dore, E.K., Kaplan, H.S., Akcay, M.M.: Radioalbumin macro-aggregates for human lung scanning, exhibit 2, J. Nucl. Med. May 1964. (abstract).

9. Taplin, G.V., Griswold, M.L., Johnson, D.E., Kaplan, H.S., Akcay, M.M.: Radioalbumin macro-aggregates for lung scanning (mechanism, safety and clinical experience) J. Nucl. Med. May 1964. (abstract).

10. Taplin, G.V., Dore, E.K., Johnson, D.E.: The hippuran I^{131} reno-cystogram (new techniques, interpretations and experiences). Radioaktive Isotope in Klinikund Forschung, 5:256-271, 1963, Urban and Schwarzenberg, Munich, Germany.

11. Taplin, G.V., Dore, E.K., Johnson, D.E.: Reticuloendothelial functions in man. Tracer studies with colloidal suspensions of human albumin I^{131}, Radioaktive Isotope inKlinikund and Forschung, 5:346-361, Urban and Schwarzenberg, Munich, Germany, 1963.

12. Taplin, G. V., Dore, E.K., Cockett, A.T., Johnson, D.E.: Radioisotopes in the diagnosis of renal hypertension, exhibit 1, J. Nucl. Med., May 1964 (abstract).

13. Taplin, G.V., Griswold, M.L., Johnson, D.E., Dore, E.K., Kaplan, H. S., Akcay, M.M.: Radioalbumin macro-aggregates for human lung scanning. J. Nucl. Med. May 1964. (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 aids 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 than 1μ are useful as test agents for visualizing the heart, liver, 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; Dept. of Radiology University of Heidelberg, Germany; University of Athens, Greece; University of Pisa, Italy; Institute of Cancer Therapy, Lisbon, Portugal; Institute of Cardiology, Mexico City; Catholic University, Santiago, Chile; Institute for Nuclear Studies, Sao Paulo, 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 1964

Renal Hypertension Studies - This has been continued using the renogram as an indicator of unilateral renal ischemia, and by correlating the renogram findings

with results of renal arteriography, I.V. pyelography and split function measurements. Further correlation has been obtained by direct measurements of renal blood flow and pressure at operation in patients with renal artery constrictive disease. An important finding was the demonstration that severe nephroptosis is capable of producing arterial hypertension which can be relieved by nephropexy alone. Furthermore nephroptosis is frequently associated with fibromuscular hyperplasia of the renal artery supplying the ptotic kidney. In such patients, prosthetic repair and restoration of normal flow plus nephropexy has led to relief of hypertension. In a group of 32 hypertensive patients with radiographic evidence of renal artery constrictive disease 30 had evidence of definite renal ischemia as indicated by quantitative radiorenography, whereas in two groups of patients with chronic pyelonephritis one with hypertension, the other without, the renograms indicated severe ischemia of the more affected kidney in less than half of the patients in both of these groups.

Radioalbumin Aggregates for Organ Scanning - Aggregates smaller than 1 μ are removed from the circulation by the liver and spleen. They are phagocytized by the Kupffer cells of the liver and digested with liberation of the iodine label to the general circulation. The I^{131} then concentrates in the gastric mucosa and the salivary glands provided the thyroid is previously blocked with excess iodide. These metabolic features of radioalbumin aggregates provide the physiological basis for photoscanning the liver, spleen, stomach and salivary glands.

Macro-Aggregates of Radioalbumin - Radioalbumin can be converted to aggregates of small particles measuring 5-25 μ . The large aggregates are prepared by a modified heat treatment and pH adjustment procedure and can be produced in less than one hour in any clinical laboratory from commercially available radioalbumin. The large particles, following I.V. injection, are trapped immediately in the lung capillaries during the first passage and remain in the lungs for a sufficient time to perform photoscans for visualization of this organ. The procedure appears to be entirely safe because toxic doses exceed those needed for scanning purposes by a factor of at least 1000.

Estimation of Liver Blood Flow and Phagocytic Function of the Kupffer Cells - Both of these functions may be estimated by measuring the blood disappearance rate of small radioalbumin aggregates following a single rapid injection. This method provides qualitative indices of flow and function. Currently the same test material is being used by constant I.V. infusion in an attempt to obtain the same information in a more quantitative fashion by applying the standard clearance principle.

15. EXPECTED RESULTS IN FY 1965

The renal hypertension studies will be continued with emphasis on obtaining further data relating direct measurements of flow with the renogram at the time of operation.

The lung scanning program will be expanded to gather information on optimal techniques and test agents in an attempt to reduce the time (60 minutes) now needed to complete the procedure. By this time we hope to be using a photoscanner adapted for I^{125} detection so that counting efficiency is increased by a factor of 10 which should greatly reduce scanning time. Studies will include

large numbers of patients without known lung disease to establish normal patterns in relation to different scanning techniques to serve as baselines for evaluating the scan patterns found in patients with pulmonary disease.

The RES function and liver spleen photoscanning program will be accelerated because by this time test material with uniform particle size distribution should be available. Finally sufficient data from toxicity studies in animals should be made available to justify initiation of kidney and brain scanning in man with large albumin aggregates as an adjunct to standard angiography.

16. EXPECTED RESULTS IN FY 1966

The projects on scanning the lungs, kidneys and brain will be continued, to incorporate technical modifications derived from related basic studies and to increase the experience with these applications of radioisotope scanning. Correlative investigations in patients will be made to evaluate their diagnostic efficiency and their clinical significance. Specifically, on the lung studies, correlative work will be done with the chest surgeons, and radiologists in the area of early detection of lung cancer by combined scanning and radiographic surveys of heavy smokers. The brain scanning investigations in primates should be well along to begin clinical studies, provided the risk of micro-embolization proves small enough to insure a wide margin of safety for clinical application. Animal toxicity studies on the safety of renal scanning should be completed by this time, so that clinical trials may be initiated. The tracer studies in liver and RES disease will be continued, with emphasis placed on developing improved methods for estimating liver blood flow, and a new technique for measuring the intra and extra hepatic shunting of blood in cirrhosis.

Renal hypertension studies will become more sophisticated because of our basic studies in animals in FY 65 with simultaneous measurements of individual renal artery blood flow and pressure together with direct measurements of renal extraction efficiency during constant sodium hippurate I-131 infusion.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	3	3½	4
(b) Other Tech.		1	1
Total	3	4½	5

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 19,900	$ 33,000	$ 40,700
(b) Materials & Services	5,900	6,800	7,200
(c) Indirect Expenses*	(2%) 21,900	(2%) 22,200	(3%) 34,000
Total	$ 47,700	$ 62,000	$ 81,900

* 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

1. Dore, E. K., Taplin, G. V., Cockett, A. T., Johnson, D. E.: Experimental basis of quantitative radiorenography for evaluating renal ischemia. J. Nucl. Med., May 1964 (Abstract).

2. Taplin, G. V., Johnson, D. E., Dore, E. K., Kaplan, H. S.: Lung scans and regional pulmonary blood flow in man using macro- radioalbumin aggregates ($<15\mu$). IAEA Symp. on Med. Radioisotope Scanning, Athens, Greece, April 20-24, 1964.

3. Taplin, G. V., Kennady, J. C., Griswold, M. L., Ackay, M. M., Johnson, D. E.: Albumin I^{125} macro-aggregates for brain scanning (experimental basis and safety). J. Nucl. Med., May 1964, (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 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 flow meters 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, Long Island, New York; Veterans Administration Center, Los Angeles.

14. TECHNICAL PROGRESS IN FY 1964

Studies on renal artery constriction in rabbits and dogs have provided the experimental basis for interpretating the slope of the second segment of the renogram as a reliable indicator of renal ischemia. These studies show that no change occurs in the renogram contour or in direct measurement of renal blood flow until a relatively severe degree of renal artery constriction is imposed by Goldblatt clamp. Further constriction causes a precipitous fall in pressure and flattening of the renogram. Release of the constriction to a degree just less than the critical level is followed by normal flow and renogram contour.

Experimental Basis for Lung Scanning: Experiments in large numbers of rabbits and dogs with albumin aggregates of mean size between 5 and 15 μ showed that the lungs can be readily visualized immediately after injection. Toxicity studies indicate that both species of animals tolerate 15 μ sized particles in doses of 20 mg/kg without pulmonary symptoms or histological evidence of capillary damage. Dogs withstand doses in the range of 20-100 mg/kg of the same size material without reactions or changes in their radiocardiogram patterns. With high specific activity radioalbumin, lung scans may be performed in man using as little as 1 mg of carrier albumin total dose. With commercial preparations containing about 10 μc/mg, the total carrier dose is only 10-15 mg or less than 2/10 mg/kg. Thus there is an extremely wide margin of safety.

Brain Scans with Large Radioalbumin Aggregates: Studies in dogs showed that the optimal particle size range for temporary entrapment in the brain capillaries is 20-70 μ. Injections were made into the internal carotid arteries. The hemisphere on the side of injection was readily visualized and could be scanned several times during the first few hours post injection. Similar studies were made in monkeys and baboons whose brain anatomy is more like that of man. Both species of animals have been able to tolerate total carrier doses of 10 mg or less without overt neurological damage or changes in their electroencephalogram patterns. Studies of relative flow to the normal vs the injected hemisphere are in progress using the hippuran I^{131} cerebrogram procedure.

15. EXPECTED RESULTS IN FY 1965

The brain scanning program will be continued to insure the complete safety of injecting particles this size using a large number of primates prior to initiating clinical investigations in patients with metastatic brain tumors.

Similar scanning and dynamic external monitoring studies will be initiated to determine the feasibility and safety of scanning the kidneys following intra arterial injection of large albumin aggregates (20-70 μ).

The renal blood flow and blood pressure experiments will be continued and in a more sophisticated manner by using electromagnetic flowmeters and transducers. Measurements obtained in this fashion are to be compared with changes in the renogram and with direct flow measurements following renal vein cannulation. Studies will be conducted in dogs to more accurately

determine the effects of renal artery constriction on flow and to determine the optimal degree of constriction which produces sustained hypertension.

16. EXPECTED RESULTS IN FY 1966

No fundamental change in scope is anticipated. Basic studies will be continued in the same general areas of renal hypertension, kidney, lung, brain and liver scanning. Emphasis will be placed on mechanism studies including radioautography with I^{125} labeled tracers at the cellular level, electromagnetic measurements of blood flow, electroencephalography, radiochromatography of tracer degradation products and development of new and/or improved tracer test materials.

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 - 1964

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

6. Working Location:
Harbor General Hospital

7. Person in Charge:
George V. Taplin, M.D.

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	1½	1½	1½
(b) Other Tech.	½	½	½
Total	1	1½	1½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 12,100	$ 16,500	$ 17,500
(b) Materials & Services	5,000	9,000	10,000
(c) Indirect Expenses*	(1%) 11,800	(1%) 11,100	(1%) 11,400
Total	$ 28,900	$ 36,600	$ 38,900

* 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

1. Johnson, D. W., Liu, C. K., Akcay, M. M., Taplin, G. V.: A lung dilution curve and the radiocardiogram in the study of the arterial and venous subdivisions of the pulmonary circulation. Proc. ORINS Symp. in Med. #8 (in press).

2. Akcay, M. M., Johnson, D. E., Taplin, G. V.: Estimation of stroke volume by steady state radiocardiography. Clin. Res. 12:103, 1964 (abstract).

3. Taplin, G. V., Dore, E. K., Johnson, D. E.: Hepatic blood flow and reticuloendothelial system studies with radiocolloids. Proc. ORINS Symp. in Med. #8 (in press).

4. Kennady, J. C., Johnson, D. E., Taplin, G. V.: A study of three clinical techniques in evaluating the anterior intracerebral vascular pattern. The American Surgeon, 29: 672-682, 1963.

5. Johnson, D. E., Kennady, J. C., Taplin, G. V.: Comparison of cerebral angiograms, electroencephalograms and radioisotope cerebrograms in evaluating intracerebral abnormalities. J. Nuc. Med. (in press)

12. SCOPE OF THE PROGRAM

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 definite 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. Caroline, by Dr. Quinn.

14. TECHNICAL PROGRESS IN FY 1964

Considerable progress has been made in this area as evidenced by the publications during the past year. Further clinical experience with the radioisotope cardiocerebrogram was accumulated through correlative studies in 160 patients in whom the results of the cerebrogram angiogram and EEG were compared. The cerebrogram continues to show promise as a simple means for detecting reduction in flow through the middle cerebral artery and is especially useful in directing the neurosurgeon to perform angiography first on the affected side.

Steady State Radiocardiography: Radiocardiography was first developed by Prinzmetal in 1948 as an external method to obtain information concerning transit times within the cardiopulmonary circulation. In this method a single rapid I.V. injection of radioalbumin is made and changing levels of radioactivity are registered by a precordial detector as the bolus first passes in and out of the heart.

The proposed method was developed through studies in phantoms and animals. Precordial radioactivity levels are registered continuously following an I.V. injection of the radioalbumin but after the blood concentration has reached an equilibrium. Such a tracing shows regularly changing levels with each cardiac cycle which are inversely related to a separate and simultaneous tracing of the carotid pulse. The onset and end of each systole can be identified. The difference in count rates at these intervals is an index of stroke volume. The major advantage of the new method is that it permits one to make repeated measurements for several hours following a single injection of tracer. In this way various agents which alter cardiopulmonary hemodynamics may be studies from an experimental viewpoint and the changes produced by various diseases of the heart and lung can be classified.

The Radiocardiogram and Lung Dilution Curve: A double tracer technique was developed for measuring the arterial and venous subdivisions of the pulmonary circulation separately by a lung dilution curve registered simultaneously with the standard radiocardiogram. The combined procedure indicates the relative magnitude of the two components of the central

circulation. In cardiopulmonary disease the magnitude of one or the other divisions is altered and in a way compatible with the patient's disease and functional status. In pulmonary congestion the venous fraction is increased whereas in pulmonary hypertension it is reduced.

15. EXPECTED RESULTS IN FY 1965

Cerebral Hemodynamics and Brain Scanning: Experimental studies in primates are to be expanded to gain better understanding of the effects of microembolization on cerebral blood flow and on potential upper neurone damage. The use of large (20-70μ) aggregates of radioalbumin for brain scanning will be investigated thoroughly in monkeys to evaluate possible detrimental effects. It is likely that a relatively large safety factor exists. If this can be proved, brain scanning with I^{125} labeled particles could be performed in man in conjunction with routine cerebral angiography. The advantages would be in the clear delineation of avascular lesions at the brain surface and a tremendous reduction in radiation exposure to the brain and other organs as compared with current methods using Hg compounds which produce excessive radiation exposure to the kidneys. To evaluate the safety of the large particle scanning technique relative flow to the two cerebral hemispheres will be studied with electromagnetic flowmeters and with the radiocerebrogram. Possible brain cell damage will be evaluated by electroencephalography by histological examination and by intelligence testing.

The clinical and primate cerebrogram studies using I^{125} and multiple probes will be continued to develop improved techniques for measuring relative blood flow through the anterior, middle and posterior cerebral arteries of each hemisphere. The program on cardiopulmonary hemodynamics will be continued with emphasis on mechanism studies in animals and clinical application in selected patients.

16. EXPECTED RESULTS IN FY 1966

The cerebral hemodynamics studies in monkeys will be continued in conjunction with the photoscanning program to correlate functional disturbances with structural defects as produced surgically. In this manner we hope to improve both the techniques for brain scanning and for measuring cardiopulmonary hemodynamics. Likewise the animal and clinical studies of cardiopulmonary hemodynamics and detection of disturbed regional pulmonary flow by photoscanning techniques will be continued. Emphasis will be placed on correlation of tracer findings with other diagnostic procedures such as electrocardiography, radiocardiography, pulmonary angiography and with gross microscopic histology whenever possible.

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 - 1964

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 1964	FY 1965	FY 1966
(a) Scientific	5	4½	5
(b) Other Tech.	1½	1½	1½
Total	6½	5 3/4	6½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 50,100	$ 54,300	$ 60,900
(b) Materials & Services	11,000	9,800	10,800
(c) Indirect Expenses*	(4%) 42,200	(4%) 44,400	(4%) 45,400
Total	$ 103,300	$ 108,500	$ 117,100

* 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 1964

1. MacDonald, N.S., Hutchinson, D.L., Moyer, D., Chez, P.: Gamma emitting radionuclides in newborns, infants and children. Science 141:1033-1035,1963.

2. Figueroa, W.G., MacDonald, N.S., Tuttle, S.G.: Calcium-47 absorption studies in man. Clin. Res., 11:110, 1963.

3. Urist, M.R., MacDonald, N.S., Moss, M.J. and Skoog, W.A.: Rarifying disease of the skeleton: Observations dealing with aged and dead bone in patients with osteoporosis. Mechanisms of Hard Tissue Destruction, pp. 385-446 publication No. 75 of the Amer. Assoc. for Advancement of Science (1963), R.F. Sognnaes, editor,

4. MacDonald, N.S., Urist, M.R., Ibsen, K.H.: Effect of tetracycline on retention of calcium and strontium in rodents. Proc. Soc. Exptl. Biol. and Med. (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 the 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 state; 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 1964

Demonstrated for the first time the rapid transport of calcium across the primate placenta from fetus to mother. This normal retrograde movement goes on continually and almost matches the amount of calcium which moves in the forward direction from mother to fetus. Analyses of blood and tissue samples (after simultaneous injection of Ca^{45} into the fetal umbilical vessel and Ca^{47} into a maternal vein of pregnant monkeys) clarified certain aspects of placental physiology. The quantities of calcium which cross the placenta every 24 hours greatly exceed the amount required for fetal growth. Apparent anomalies in the very early clearance of radiotracer from maternal blood compared to fetal blood were attributed to variable mixing of maternal blood in the placental intervillous space.

Developed a method for estimating the efficiency of absorption of calcium in humans by administering an oral dose of calcium labeled with Ca^{47} and repeatedly measuring the radioactivity in the subject over a period of a week or more, using the Total Body Counter. Data on absorption and retention of calcium by 10 patients were presented. The method avoids the onerous task of collecting and analyzing excreta.

15. EXPECTED RESULTS IN FY 1965

Collaborative efforts with the university departments of Radiology, Medicine, Obstetrics and the Veterans Administration Hospital (Sawtelle) will be continued. Some of this work begun in FY 1963 will be completed. Studies of the efficiency of oral absorption and retention of calcium in patients, using the Total Body Counter and radioactive Ca^{47} will be continued. Studies of the early deposition and long time retention of Strontium-85 and Calcium-47 in 26 patients with disorders of skeletal metabolism, such as osteoporosis, will be reported. Investigations of the body retention of serum albumin (and possibly other proteins labeled with radioiodine) in patients with various diseases will be continued. Experimental studies of the transfer of radioactive nuclides across the placental membrane will be pursued, using animals. The monitoring of infants, children and adults for body burdens of gamma-emitting fission products will continue.

16. EXPECTED RESULTS IN FY 1966

Investigations 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, chloride and possibly phosphate from the blood soon after injection will be studied using multiple radioactive tracers, circulating blood loops and fast recording on multi-channel magnetic tape. This information will be pertinent to certain clinical problems which may involve disorders in the homeostatic control of these electrolytes. Efforts to reduce the detrimental effects of accumulation of radionuclides within the body (particularly radiostrontium and radiocesium) will continue. Collaborative programs of clinical research utilizing radioisotopes with other departments of the medical center will be intensified. For example, the number of patients in whom $Iron^{59}$ retention has been studied with the Total Body Counter Facility should reach about 50 and will be reported. Our knowledge of the physiological

behavior of bone seeking radionuclides such as Ca^{47} and Sr^{85} during the first hour after entry into the blood will have advanced so that our interpretaion of the radioisotope osteograms in patients with skeletal disorders will become less empiric. The Total Body Counter Facility will continue its service functions of monitoring people for gamma emitting fission products and for emergency measurements of persons in local and nearby communities who are accidentally exposed to radioisotope contamination.

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.:	4. Date Prepared:
06-10-00	April - 1964
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 1964	FY 1965	FY 1966
(a) Scientific	3½	3½	3½
(b) Other Tech.	-	-	-
Total	3½	3½	3½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 35,300	$ 45,100	$ 48,400
(b) Materials & Services	1,000	4,000	4,000
(c) Indirect Expenses* (3%)	32,100	(3%) 33,300	(3%) 34,000
Total	$ 68,400	$ 82,400	$ 86,400

* 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

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. These studies have been concerned chiefly with the erythropoietic and reticuloendothelial systems. Our total body counter is used in these studies, and in investigations of the metabolism of radionuclides of clinical interest (e.g., radiozinc, radioiron).

13. RELATION TO OTHER PROJECTS

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 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, L.I., N.Y.

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 of the Department of Chemistry, UCLA.

14. TECHNICAL PROGRESS IN FY 1964

(1) Two manuscripts reporting studies of zinc and iron metabolism have been prepared for publication: (a) The Metabolism of Radioactive Zinc-65 in Patients with Neoplastic Disease, (b) The Effect of Inflammation on the Utilization and Retention of Transferrin Bound and Hemoglobin Radioactive Iron.

(2) Because of the rapid and marked increase in atmospheric C^{14} during the past two years, the rate of accumulation of C^{14} in human tissues is of interest. In collaboration with Dr. J. Mead and Mr. G. Alexander of the Laboratory of Nuclear Medicine and Radiation Biology and Dr. W. F. Libby of the UCLA Department of Chemistry, we have begun studies of the C^{14} content of human tissues obtained at different times during the past two and a half years.

The concentration of C^{14} in various chemical fractions of these tissues are being compared with the atmospheric content of C^{14} known to have existed at the time this tissue was removed from its donor. The complex relationships existing between tissue C^{14} concentration and atmospheric concentration are under study.

(3) Complexities in the administration of the Laboratory of Nuclear Medicine and Radiation Biology occupied the major portion of the efforts of the scientist in charge of this project who also serves as Director of the Laboratory.

(4) Instruction in Nuclear Medicine, Radiation Biology, and Biophysics to medical students, residents, graduate students, and postdoctoral fellows was advanced effectively during this period. The teaching and training potentialities of the Laboratory of Nuclear Medicine and Radiation Biology have begun to be realized. During 1963-64, 21 graduate students, 10 medical students, and 20 postdoctoral trainees have been included in the programs of the Department and Laboratory, and instruction in subject areas related to Contract GEN-12 is progressing very well.

15. EXPECTED RESULTS IN FY 1965

(1) Studies of the effects of ionizing radiation on ferrokinetics, on erythropoiesis, and on the relationship of the reticuloendothelial system to these functions will be undertaken, employing the techniques and procedures developed during recent years. Additionally, we plan to reinstitute studies of the influence of radiation and inflammation on red blood cell and hemoglobin formation in human subjects. Studies of zinc metabolism in human subjects, employing radiozinc, the total body counter, and the analytic techniques of emission spectrography will be continued.

(2) Predoctoral and Postdoctoral training of graduate students and physicians in research application of radioisotope tracer techniques will be emphasized.

16. EXPECTED RESULTS IN FY 1966

(1) Increasing emphasis will be placed on radioisotope tracer studies of 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 reticuloendothelial and erythropoietic systems.

(2) Studies of metal containing tissue enzymes and the effects of ionizing radiation on their concentration and function will be initiated.

(3) Use of the total body counter for studies of the metabolism of radiozinc and radioiron will be emphasized.

(4) Predoctoral and Postdoctoral training in research in Nuclear Medicine will be expanded.

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 - 1964

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

6. Working Location:
UCLA

7. Person in Charge:
Thomas G. Hennessy, M.D.

8. Project Term:
From: 1954 To: Continuing

9. Man Years	FY 1964	FY 1965	FY 1966
(a) Scientific	3½	3	3½
(b) Other Tech.	-	-	-
Total	3½	3	3½

10. Costs	FY 1964	FY 1965	FY 1966
(a) Direct Salaries	$ 25,200	$ 32,100	$ 38,700
(b) Materials & Services	3,500	4,000	4,000
(c) Indirect Expenses*	(2%) 21,900	(2%) 22,200	(3%) 34,000
Total	$ 50,600	$ 58,300	$ 76,700

* 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 1964

1. Evaluation of Erythropoietic Damage and Recuperation Following X-Irradiation. T. G. Hennessy, S. E. Herrick and J. P. OKunewick. Health Physics, Vol. 10, pp. 49-53 (1964).

2. Effects of Plasma on the Absorption of Zinc by Mammalian Erythrocytes. J. P. OKunewick, S. E. Herrick and T. G. Hennessy. In Press J. Cellular and Comparative Physiology (1964).

3. Synthesis of Cytoplasmic Hematin by Nuclei of Erythrocytes from Embryos. O.A. Schjeide, G. V. Alexander, J. P. OKunewick, C. R. Carmack, M. Wilkins, E. N. Carlsen and T. G. Hennessy. Submitted to Growth (1964).

4. Effect of X-Irradiation on the In Vivo Uptake of Zn^{65} by Rat Blood Cells. J. P. OKunewick, S. E. Herrick and T. G. Hennessy. Submitted to Radiation Research (1964).

12. SCOPE OF THE PROJECT

Recent studies in iron metabolism using radioactive iron as a tracer have provided valuable diagnostic tools for evaluating the extent of accidental radiation injury and also for the study of pathological conditions not usually associated with radiation injury. In addition, radioactive iron has proven to be of value in basic investigations of the erythropoietic mechanism, especially as related to red cell formation following irradiation damage. The present studies are continuing along these lines.

Studies in zinc metabolism have heretofore not been concentrated on irradiation effects, due to certain problems inherent to the normal uptake and retention of the zinc isotope. Techniques recently developed in this laboratory have overcome some of these problems and are currently being applied to study the effects on the carbon dioxide transport system.

13. RELATIONSHIP TO OTHER PROJECTS

Similar work on Zn^{65} metabolism is being carried out at Hanford Atomic Products Operations, 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 1964

One of the major accomplishments during this year was the completion of and publication of a paper on the interrelationships of irradiation dosage and the time of radioactive iron administration on radioiron uptake curves in animals. It was demonstrated that radioiron uptake curves could be used to demonstrate both the amount of irradiation received by the animal and to predict the ability of the animal to recover from the dosage received.

Another study was begun using radioiron assay of erythropoietin. The presence of erythropoietin was shown to be present in a certain class of human brain tumors, cerebellar hemangioblastomas, which stimulate red cell production, and shown to be absent in cerebellar brain tumors of two other types.

Work continued on Zinc metabolism using Zn^{65} tracer. In vitro investigations show the existence of a competitive system involving the plasma and red cells with regard to the binding of radiozinc. In vivo investigations of Zn^{65} binding by the cellular components of rat blood following total body irradiation have shown a depression in zinc uptake of approximately 20 per cent.

15. EXPECTED RESULTS IN FY 1965

Study of the depression of erythropoiesis by irradiation will continue during the year. There will be increasing emphasis on the study of erythropoietin and irradiation injury to determine if there is any manner in which this substance can be used to alleviate irradiation injury. It is expected that during this year mice will be raised in an altitude chamber to produce plethoric mice which will be used for Fe^{59} erythropoietin assay rather than the present system of employing hypertransfused animals, a development which should accelerate these studies. The additional studies of erythropoietin in human cerebellar tumors will continue as cases become available and an attempt will be made to determine which cells or areas of the tumor are producing the hormone.

Zinc metabolism studies using Zn^{65} will continue with particular emphasis on the production of the zinc containing respiration enzyme, carbonic anhydrase, in normal and irradiated animals. Present studies indicate that irradiation may probably influence the production of this enzyme.

16. EXPECTED RESULTS IN FY 1966

No major change in size or scope of the program is anticipated during this year. However, there will be shifts of emphasis depending upon the previous year's work. At the present time it is anticipated that the zinc studies will be concluded or at least de-emphasized and greater emphasis will be placed on the expanding erythropoietin studies. One of the major studies anticipated during this year is whether erythropoietin has any relationship to myelopoiesis. Two of the studies planned for this year are an attempt to stimulate myelopoiesis in the plethoric mouse with erythropoietin following irradiation injury and an attempt to prevent leukemogenesis in mice by suppressing erythropoiesis. It is also anticipated that the cerebellar tumor studies which were started two years ago should be nearing conclusion.