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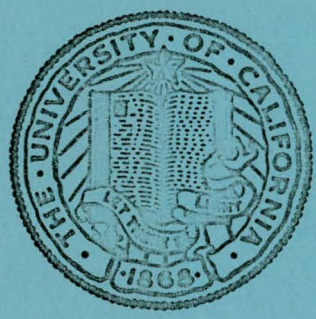

Form AEC-189's

RESEARCH AND DEVELOPMENT PROGRAM

FISCAL YEAR 1970

April 1968

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 1970

APRIL 1968

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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

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; 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 systems of biological significance and on living organisms.

2. Assessment and study of the immediate and long term consequences of the environmental radioactivity on flora, fauna, and man.

3. Development of beneficial uses of ionizing radiation and radioactive substances in medicine and biology.

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

The administration of the University of California, Los Angeles, and the Division of Biology and Medicine, AEC, have recently agreed on the nomination of O. R. Lunt as Laboratory Director, and it is assumed that the appointment will be confirmed in due course by the Regents of the University and the Commissioners of the AEC. It is anticipated that considerable review and evaluation of individual projects will be conducted in the next few months and that there will be some reorientation of existing programs, as well as the introduction of new programs, over the next two or three years. Certain shifts of emphasis within the overall objectives of the Laboratory have already become apparent, resulting in corresponding changes in the level of support among the various categories of the Biology and Medicine Program and it is probable that additional shifts will occur in the future. However, at this early stage of administrative change and the formulation of long-range research objectives, it is impossible to project with any accuracy the specific categories that may be involved.

At the present time it is expected that the programs in the Laboratory's Nuclear Medicine and Environmental Radiation divisions will be strengthened during FY 1969 and FY 1970, and that new programs will be introduced in the general field of developmental biology. The availability of a cyclotron specifically designed for biomedical investigations (previously requested for installation during FY 1969) will permit a significant expansion of effort in these areas and will also be a powerful tool in current studies in radiation biology and radiation chemistry.

Based upon these tentative plans, it appears that there will be an increase of research activity during FY 1969 and FY 1970 in the categories of Terrestrial and Fresh Water Ecology and in Selected Beneficial Applications.

Re-evaluation and reorientation of programs within the category of Molecular and Cellular Level Studies will result in a temporary reduction of effort during FY 1969, but with a return to previously forecast levels during FY 1970 as a result of the introduction of the new studies in developmental biology.

A slight decrease will occur in the category of Radiological and Health Physics during FY 1969 pending completion of current review and re-evaluation of the program.

It is anticipated that all other categories will be continued at approximately their current levels of effort during FY 1969 and FY 1970.

The composition of costs and staffing for FY 1968, FY 1969, and FY 1970 are summarized below for the entire Biology and Medicine Program by major categories of expense.

	FY 1968	FY 1969	FY 1970
I. Costs: (Shown in Thousands)			
Salaries and Burden (Direct)	$1,328.6	$1,413.5	$1,556.5
Supplies, Travel, & General Expense	218.4	229.7	263.3
Indirect Costs	993.0	1,006.8	1,080.2
Total Operating Costs	$2,540.0	$2,650.0	$2,900.0
II. Manpower: (Shown as Full Time Equivalence)			
Direct Man Years	118	120	129
Direct Scientific Man Years	103	105	114
III. Cost Per Man Year Data: (Shown in Thousands)			
Cost per Direct Man Year	$ 21.9	$ 22.1	$ 22.5
Cost per Direct Scientific Man Year	$ 24.7	$ 25.2	$ 25.4

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 proration indirect costs are not specifically identifiable under individual research projects, and for this reason, the composition of indirect expense for the total Program is given below in some detail.

	FY 1968	FY 1969	FY 1970
Composition of Indirect Expense:			
I. Manpower (Shown as Full Time Equivalence):			
Administrative Services	36	34½	36
Technical Services	16	14½	16
Building Maintenance	7	7	7
Total Indirect Personnel	59	56	59
II. Costs (Shown in Thousands)			
Administrative Services	$ 397.6	$ 393.6	$ 439.2
Technical Services	208.4	206.9	222.7
Building Maintenance	75.0	80.0	85.0
Building Amortization	126.7	126.7	126.7
Utilities	70.0	75.0	75.0
U. C. Management Allowance	75.0	75.0	75.0
U. C. Accounting & Purchasing Services	32.4	34.0	36.0
Miscellaneous (Laundry, Postage, General Supplies, etc.)	7.9	15.6	20.6
Total Indirect Costs	$ 993.0	$ 1,006.8	$ 1,080.2

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 1968 COSTS	FY 1968 MAN YRS	FY 1969 COSTS	FY 1969 MAN YRS	FY 1970 COSTS	FY 1970 MAN YRS
06-01-01	Radiation Effects - General						
	Medical Physics Problems (B. Cassen)	$ 52.4	3	$ 56.1	3	$ 75.4	3 1/2
	Late Effects Radiobiology (L. Bennett)	35.6	2	37.5	2	38.8	2
	ACTIVITY TOTAL	$ 88.0	5	$ 93.6	5	$ 114.2	5 1/2
06-04-00	Molecular & Cellular Level Studies						
	Enzyme Chemistry (I. Harary)	$ 150.7	6 1/2	$ 119.5	5 1/2	$ 124.0	5 1/2
	Macromolecular Chemistry (N. Simmons)	51.6	3 1/2	80.7	4 1/2	88.8	5
	General Metabolism (J. Mead)	82.6	4	119.7	5 1/2	130.2	6 1/2
	Organic Chemistry (D. Howton)	72.1	2 1/2	74.7	2 1/2	66.6	2 1/2
	Tracer Synthesis (J. Nevenzel)	46.7	1 1/2	56.6	2 1/2	59.1	2 1/2
	Macromolecular Separation Methods (A. Kolin)	24.7	1	-	-	-	-
	Chemical Radiobiology (L. Myers)	106.7	5 1/4	109.5	5	139.6	6
	Sub-Cellular Radiobiology (J. Ward)	30.5	1 1/4	53.2	2	57.0	2
	Metabolic Radiobiology (O. Schjeide)	99.1	4	81.4	3	99.3	4
	Developmental Radiobiology (J. deVellis)	49.6	2 3/4	54.5	3	60.3	3
	Cellular Radiobiology (N. deTerra Whittaker)	19.2	3/4	-	-	-	-
	Physical Radiobiology (H. Strickland)	74.5	4	77.2	4	85.5	4 1/2
	ACTIVITY TOTAL	$ 308.0	37	$ 827.0	37 1/2	$ 910.4	41 1/2
06-05-01	Terrestrial & Freshwater Ecology						
	Soil Factors (H. Nishita)	$ 93.2	4 1/4	$ 95.8	4 1/4	$ 103.6	4 1/4
	Plant Factors (E. Romney)	97.5	4 1/4	100.2	4 1/4	106.7	4 1/4
	Environmental Factors (H. Hawthorne)	98.9	5 1/4	97.9	4 3/4	93.6	4 3/4
	Radiation Ecology (N. French)	146.4	6 1/4	147.2	6 1/4	135.9	5 3/4
	Ecology of the Nevada Test Site (J. Beatley)	72.1	3 3/4	78.6	4 1/4	82.8	4 1/4
	Radioecology (F. Turner)	101.8	4 1/4	102.1	4 1/4	109.5	4 1/4

	Physiology of Mineral Accumulation in Plants (O. R. Lunt)		30.0	1 3/4		27.7	1 1/4	40.2	2 1/4
	Radioecology - Arthropods (V. Stern)		46.3	2 1/4		33.4	1 3/4	35.6	1 3/4
	Plant Physiological Ecology (A. Wallace)		82.8	3 1/4		112.3	4 1/4	112.7	4 1/4
	Chemical Problems (R. Wood)		157.0	7 3/4		155.4	7 3/4	162.6	7 3/4
	Quantitative Plant Ecology (To Be Named)		-	-		22.2	3/4	56.5	2
		ACTIVITY TOTAL	$ 926.0	43		$ 972.8	43 3/4	$1,039.7	45 1/2
06-06-01	Radiological and Health Physics								
	Excited States of Molecules in Radiation Biology (R. Lehman)		$ 48.0	2 1/2		$ 30.8	2	$ 31.8	2
		ACTIVITY TOTAL	$ 48.0	2 1/2		$ 30.8	2	$ 31.8	2
06-06-02	Radiation Instruments								
	Medical Physics Instrumentation (B. Cassen)		$ 57.0	2 1/2		$ 58.9	2 1/2	$ 59.7	2 1/2
		ACTIVITY TOTAL	$ 57.0	2 1/2		$ 58.9	2 1/2	$ 59.7	2 1/2
06-09-00	Cancer Research								
	Leukemia Biology (E. F. Hays)		$ 75.0	4		$ 83.9	4 1/4	$ 89.3	4 1/2
		ACTIVITY TOTAL	$ 75.0	4		$ 83.9	4 1/4	$ 89.3	4 1/2
06-10-00	Selected Beneficial Applications								
	Clinical Nuclear Medicine (G. Taplin)		$ 133.5	5 1/4		$ 153.5	6	$ 150.3	6
	Basic Nuclear Medicine (N. Poe)		54.6	3 1/4		58.8	3 1/4	63.0	3 1/4
	Hemodynamics (J. Kennady)		77.4	4		55.3	2 3/4	56.5	2 1/2
	Nuclide Metabolism (N. MacDonald)		125.1	5 1/2		107.7	5	113.0	5
	Radiodiagnostic Agent Development (G.Taplin and N.MacDonald)		48.2	1 1/4		76.7	2 1/2	86.8	3
	Clinical Studies-Short Lived Isotopes (M. Webber)		46.1	2		59.1	2 1/2	86.0	3 1/2
	Biomedical Cyclotron Facility (B. Cassen and N. MacDonald)		-	-		17.0	1/2	41.3	1 3/4
	Mammalian Radiobiology (T. Hennessy)		53.1	2 3/4		54.9	2 1/2	58.0	2 1/2
		ACTIVITY TOTAL	$ 538.0	24		$ 583.0	25	$ 654.9	27 1/2
		TOTAL 06-00-00 PROGRAM-	$2,540.0	118		$2,650.0	120	$2,900.0	129

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 - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Benedict Cassen

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2	2	2 ½
(b) Other Tech.	1	1	1
Total	3	3	3 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 31,100	$ 32,000	$ 39,600
(b) Materials & Services	1,700	4,000	3,400
(c) Indirect Expenses *	(2%) 19,600	(2%) 20,100	(3%) 32,400
Total	$ 52,400	$ 56,100	$ 75,400

* 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 1968

Zucker, R. M., Cassen, B., Taplin, G., and Kennady, J. C.: The Size Control of Radioalbumin Macoraggregates in Suspension. Abstracts of Program of Seattle Meeting of the Society of Nuclear Medicine, 1967.

Poe, N. and Cassen, B.: Quantitative Scanning. Abstracts of Program of Seattle Meeting of Society of Nuclear Medicine, 1967.

12. SCOPE OF THE PROJECT

Currently, in this project studies are being made by use of new methods and techniques (see Budget Activity 06-06-02) on the effects of radiation in vivo and in vitro on mammalian blood cells, especially lymphocytes. Under certain conditions (see 14 below) size distributions and electrophoretic mobility distributions have been changed significantly by irradiation. These effects are being studied in more detail and experiments are being undertaken to prove or disprove possible explanatory hypotheses. Further evidence has been found which indicates that radiation can stimulate small lymphocytes somewhat analogously to their immunological stimulation. Also, studies are being made as to the detectability by these new methods of cell changes in the blood of cancer patients.

The new methods are also being applied to the study of bone marrow cell suspensions. Besides being able to show that the various precision density fractions of bone marrow have characteristic bimodal size distributions, it has been shown that various electrophoretic fractions of these cells also show characteristic bimodal size distributions.

13. RELATIONSHIP TO OTHER PROJECTS

Dr. M. Ingram, University of Rochester, Atomic Energy Project
Dr. C. Craddock, UCLA School of Medicine
Dr. E. Cronkite, Brookhaven National Laboratory
Dr. Marvin Van Dilla
and recently many others.

14. TECHNICAL PROGRESS IN FY 1968

The perennial problem of the clean and efficient separation of leukocytes from the overwhelming number of red cells in mammalian or human blood without affecting the viability or measurable properties of the minority population, appears to have been finally solved by a very simple procedure. Whole blood is centrifuged in a bovine serum albumin density gradient until equilibrium is attained. The leukocytes separate from the red cells in definite density bands and the lymphocytes separate from other luekocytes. The lymphocyte bands show a gradation of size distributions as determined with the multichannel analyzer Coulter aperture system. The lymphocytes can be drawn off and rebanded with higher resolution in a less steep density gradient. They maintain their viability as can be demonstrated by growing cell cultures. Also various fractions can be studied with other separation or measurement procedures. A paper is being prepared describing the size distributions of various density fractions.

It was found that the size distribution determination system showed that some of the red cells in new-born mice had over twice the volume of that of adult red cells. It is well known that in the embryo, red cells are first formed in the liver and spleen before being formed in the bone marrow. A small scale study is being made to use the available size distribution determining system to study the time course of red cell volume changes prenatally and immediately post-natal in the blood of mice foetuses and in the new-born.

The Hematology group of the UCLA Department of Medicine has shown a renewed interest in a cooperative program to apply our techniques (density gradient, electrophoresis, size distribution, etc.) to the study of blood from patients with hematological diseases and with cancer. This program is getting under way.

The multichannel analyzer size distribution system has been used successfully to demonstrate the size distribution of macroaggregated albumin. The break up of a tail end of large particulates by ultrasound can be readily demonstrated.

15. EXPECTED RESULTS IN FY 1969

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

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

16. EXPECTED RESULTS IN FY 1970

It is anticipated that in FY 1970 there will be a continuation of the development of techniques and methods for measuring properties of lymphocytes and that these will be applied to the study of the complex physiology and radiation biology of these cells. It is expected that a program will be initiated to determine the effects in vitro and in vivo of various agents such as certain pharmacologically active agents, polypetides, etc., on cell size distribution and electrophoretic mobility. It is expected that cell size distribution and electrophoretic mobility changes can be observed in animals and in human blood in various disease states.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

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

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

2. Project Title:
Late Effects Radiobiology

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Leslie R. Bennett, M.D.

8. Project Term:
From: 1950 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2	2	2
(b) Other Tech.	-	-	-
Total	2	2	2

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 19,200	$ 19,700	$ 19,800
(b) Materials & Services	6,500	7,700	8,200
(c) Indirect Expenses *	(1%) 9,900	(1%) 10,100	(1%) 10,800
Total	$ 35,600	$ 37,500	$ 38,800

* 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 1968

Webber, Milo M.: Allograft Survival Following Antibody Suppression with Radioiodine-Labeled Antigen, Transplantation 5:4:2:1198-1203 (1967).

Lamson, Baldwin G., Billings, Marta S., and Bennett, Leslie R.: Incidence of Disease and Cause of Death Following Single and Divided Doses of Total Body X-Irradiation in Rats, UCLA Report to United States Atomic Energy Commission 12-616 (1967).

Bennett, L. R. and Connon, F. E.: An Additive Response of Ehrlich Ascites Tumor in Vivo to Gamma Radiation Combined with DL-Glyceraldehyde or Methylglyoxal, Rad. Res. (In Press, April 1968).

12. SCOPE OF THE PROJECT

The purposes of the research of this section are to study 1) the diseases and longevity observed in previously irradiated animals, and 2) the effect of local internal irradiation on both the immediate and delayed responses of the immune system. Many variables such as age, sex, total irradiation dose, fractionation and spacing of irradiation dose, and portion of the body irradiated may influence longevity and type of pathology observed. Studies are required to systematically assess the influence of these various factors in order to more properly define the conditions of exposure to ionizing irradiation 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 and the problem of tissue transplantation can also be expected.

13. RELATIONSHIP TO OTHER PROJECTS

No other project in the 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 are the following: Storer (Bar Harbor); Mole (Harwell); Hursh, Casarett (Rochester); Busted (UC-D); Kaplan (Stanford); Conard (Brookhaven); Upton (Oak Ridge). Studies on heavily labeled antigens are related to projects under Campbell (Cal Tech).

14. TECHNICAL PROGRESS IN FY 1968

Based on the previously reported work regarding antibody suppression following irradiation in rabbits with intravenous injection of particulate antigen labeled with radioiodine-131, work has continued in the investigation of the characteristics of transplantation antigen as a possible carrier for heavy doses of radioiodine-131 in order to achieve antibody suppression. Initial work has centered around the technique of separation of transplantation antigen from mouse spleens. This has followed the method of Al-Askari, et al., and involves maceration of the spleens followed by an ultra-centrifugation fractionation of the spleen components. Transplantation antigen appears to be centered primarily in the microsomal fractions. Radioiodine tagging of this material utilizing radioiodine-131 and radioiodine-125 has been performed following the iodine monochloride method as suggested by Helmkamp

and associates. Blood clearance studies using tagged antigen have been performed, and suggest primary uptake of the tracer within the reticuloendothelial system. Gross organ localization studies have also been carried out, substantiating this finding. Microscopic localization studies utilizing the techniques of autoradiography with the antigen tagged with radioiodine-125 are currently under way.

Studies on late effects of irradiation have been continued using salt stress to measure the capacity of irradiated rats to do metabolic work. On chronic salt stress the animals become hypernatremic, a condition they tolerate for weeks or months. This is followed by a state of metabolic exhaustion characterized by marked weight loss, a general cachexia, and a drop in oxygen consumption. The depression of oxygen consumption in these animals under a metabolic work stress has been investigated in some detail during the past year. The enzymes of the cytochrome chain appear to be intact and capable of normal function. The fall in oxygen consumption is now assumed to be distal to the respiratory enzymes and to involve a lack of coenzymes, substrate, or normal regulatory control of oxygen utilization.

DL-Glyceraldehyde has been shown to have an additive effect to gamma irradiation in Ehrlich ascites tumor in mice. This additive effect has not been demonstrated in normal tissues. DL-Glyceraldehyde appears to interfere with repair mechanisms since the effect can be demonstrated as long as 90 minutes after irradiation.

15. EXPECTED RESULTS IN FY 1969

Studies on the distribution and fate of transplantation antigens will be continued using I-131 and I-125 tags. Microscopic localization studies using radioautography will be continued. It is hoped these studies will enable us to have an understanding of the fate of transplantation antigen when used as a carrier for radioisotope tracers. In addition to the above studies, the giving of repeated doses of heavily labeled transplantation antigen will be followed for effect on longevity, incidence of infection, and possible induced pathology.

Late effects studies on salt stressed irradiated animals will be continued. It is expected that these studies will lead to a clarification of the nature of the defect in oxidative metabolism in these animals. In addition, the studies should yield basic information on regulation of oxidative metabolism, and the metabolic changes associated with natural aging.

The studies on DL-Glyceraldehyde will also be continued with experiments aimed at elucidating the mechanism of the additive response with gamma irradiation, and the possible exploitation of this effect as an adjunct in radiation therapy.

16. EXPECTED RESULTS IN FY 1970

Animals receiving repeated doses of heavily labeled antigen will continue to be followed for late effects. New experiments will include a study of the additive effect of heavily labeled antigen and overloading with transplantation antigen on mouse skin and rat kidney transplants.

Studies using the irradiated salt stressed rat and appropriate controls

will be continued to demonstrate the metabolic defects produced by irradiation. Experiments will be designed to obtain information on the capacity to synthesize protein, nucleic acids, fats, and carbohydrates under stress. These studies should also give information on the regulatory mechanisms of these synthetic processes in normal animals.

The DL-Glyceraldehyde studies will be followed by a study of the effects of other short chain aldehydes (e.g., methylglyoxal) on irradiation sensitivity of tumor cells.

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 - 1968

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 1968	FY 1969	FY 1970
(a) Scientific	5 ½	4	4
(b) Other Tech.	1	1 ½	1 ½
Total	6 ½	5 ½	5 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 74,300	$ 60,300	$ 60,700
(b) Materials & Services	16,700	18,900	20,100
(c) Indirect Expenses *	(6%) 59,700	(4%) 40,300	(4%) 43,200
Total	$ 150,700	$ 119,500	$ 124,000

* 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 1968

J. F. Mead, D. F. Haggerty, L. E. Gerschenson, and I. Harary. Recent Advances in Polyunsaturated Fatty Acid Metabolism. Biochem. Pharm. 50, 1 (1967).

I. Harary. Reversible Changes of Specific Function in Beating Heart Cells in Culture. Chapter in Carcinogenesis: A Broad Critique. Published for The University of Texas M.D. Anderson Hospital and Tumor Institute, Houston, Texas by The Williams and Wilkins Co., Baltimore, Maryland, 1967.

I. Harary, L. E. Gerschenson, D. F. Haggerty, Jr., W. Desmond and J. F. Mead. Fatty Acid Function in Cultured Heart and HeLa Cells. Chapter in Lipid Metabolism in Tissue Culture Cells, G. H. Rothblat and D. Kritchevsky, Eds., Wistar Press, 1967.

I. Harary, Maria Seraydarian, and L. E. Gerschenson. Effect of Lipids on Contractility of Cultured Heart Cells. Chapter in Factors Influencing Myocardial Contractility, R. D. Tanz, F. Kavaler, and J. Roberts, Eds., Academic Press, New York, 1967.

12. SCOPE OF THE PROJECT

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

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

13. RELATIONSHIP TO OTHER PROJECTS

The following workers in other laboratories are investigating similar problems:

H. Green, The Synthesis of Cartilage Precursors by Cells in Culture; E. Goldwasser, The Effect of Erythropoietin in Inducing Hemoglobin Synthesis in Erythroblasts in Culture; Rabinowitz, The Synthesis of Myosin in Sub-Cellular Heart Fractions; Evans et al., Relation of Lipids to Heart Function; The Cloning of Chick Heart Cells, Cahn et al.; The Myogenesis and Cell Division in Skeletal Cells, H. Holtzer; Myogenesis in Culture, Konigsberg.

14. TECHNICAL PROGRESS IN FY 1968

The purpose of our study of single heart cells in culture is to determine

the relation of specific metabolism to specific function.

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

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

The effect of lipids could be due to:

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

2. The role of lipids in membrane function.

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

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

(1) The investigation of the sources of energy for the maintenance of ATP in the heart cell. We have underway an analysis of the role of the three sources of energy for ATP synthesis, oxidative phosphorylation, glycolysis and creatine phosphate transfer. All three are used to maintain ATP. When either glycolysis or oxidative phosphorylation is inhibited, creatine phosphate decreases drastically within 15 minutes and the ATP level continues at a somewhat decreased value maintained only by one source left.

(2) The control of fatty acid oxidation and oxidative phosphorylation for the maintenance of ATP is now being investigated directly by examining the oxidation of fatty acids in heart homogenates and investigating the effect of glycolysis and the products of glycolysis on the rate and extent of the oxidation. The requirements for fatty acid oxidation by the heart homogenate have been determined and the shape of the curve for ATP requirement indicates that control may be exerted on the first step, the activation of fatty acids.

(3) We reported that a factor in serum controls the myosin level. We now have evidence that this factor is a lipid free serum protein. A protein fraction from fetal bovine and human serum can maintain the level of myosin without growth of the cells. This effect is not present in either the heat inactivated, or hydrolyzed, serum protein.

(4) We have also begun an analysis of the kinetic parameters of myosin synthesis in the cultured heart cells. Methods have been perfected to follow the uptake of radioactive amino acids into the free amino acids in the cell and into myosin.

15. EXPECTED RESULTS IN FY 1969

We expect to continue these projects. We expect that in the next year we will isolate the protein in the serum which affects myosin synthesis. We also expect to analyze the way the cell controls the myosin level and thus investigate how the serum protein acts to maintain the myosin level.

16. EXPECTED RESULTS IN FY 1970

We hope to begin an analyses of the control of messenger RNA synthesis for the synthesis of myosin. This will require new techniques for analyzing and separating the genetic material and also the various types of RNA. We also expect to look at the mechanism of control of myosin synthesis in an *in vitro* system.

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.:	4. Date Prepared:
06-04-00	April - 1968
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 1968	FY 1969	FY 1970
(a) Scientific	3	3 ½	4
(b) Other Tech.	½	1	1
Total	3 ½	4 ½	5

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 21,300	$ 37,200	$ 43,100
(b) Materials & Services	10,400	13,300	13,300
(c) Indirect Expenses *	(2%) 19,900	(3%) 30,200	(3%) 32,400
Total	$ 51,600	$ 80,700	$ 88,800

* 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 1968

N. S. Simmons and A. N. Glazer. An Analysis of the Tyrosine Circular Dichroism Bands in Ribonuclease. J. Am. Chem. Soc., 89, 5040 (1967).

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, lipoproteins and glycoproteins).

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

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

13. RELATIONSHIP TO OTHER PROJECTS

Related projects supported by AEC funds are too numerous to mention here. However, major programs in This area are also being conducted at Harvard (Doty, Blout), Cornell (Scheraga), Duke (Tanford) and Univ. of Calif. at Berkeley (Tinoco, etc.), Columbia (Beychok), UC San Francisco (Yang), UC LaJolla (Singer) etc.

14. TECHNICAL PROGRESS IN FY 1968

Macromolecular Structure and Function. This major investigative effort has been continued with the purpose of attempting to relate structure to function in macromolecular biopolymers such as the nucleic acids, proteins, viruses etc. by the measurement of their optical activity in the ultraviolet spectral regions. Since the function of most macromolecules is largely dependent upon specific steric relationships of active sites within or upon their surfaces (enzymes, antibodies, etc.) minor changes in the gross conformation of such molecules might be presumed to lead to the diminution or destruction of their biological activity. If these active sites involve aromatic amino acid side chain residues it might be possible to detect this by changes in optical activity in the regions of the chromophoric absorption.

As described in the above publication, the circular dichroism of ribonuclease was studied in the region of the Tyrosine bands along with a model compound Nacetyl-L Tyrosinamide. It was shown that the residues responsible for the enhanced optical activity in this enzyme are at the surface and can be described as showing normal ionization behavior. Further it was shown that exposure of tne enzyme to sodium dodecyl sulfate resulted in the elimination of the negative CD band ascribable to oriented Tyrosine residues. From this, the compelling conclusion must be drawn that electrostatic interactions play a significant role in the conformational stability of the enzyme.

15. EXPECTED RESULTS IN FY 1969

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

16. EXPECTED RESULTS IN FY 1970

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

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

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
General Metabolism

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

4. Date Prepared:
April - 1968

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 1968	FY 1969	FY 1970
(a) Scientific	3	4 ½	5 ½
(b) Other Tech.	1	1	1
Total	4	5 ½	6 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 46,500	$ 64,700	$ 70,700
(b) Materials & Services	6,200	4,700	5,500
(c) Indirect Expenses *	(3%) 29,900	(5%) 50,300	(5%) 54,000
Total	$ 82,600	$ 119,700	$ 130,200

* 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 1968

J. F. Mead and Roslyn B. Alfin-Slater. Toxic Substances in Food Fats, Chap. in Toxicants Occurring Naturally in Foods, J. M. Coon, Ed. (1967).

J. F. Mead and M. Kayama. Lipid Metabolism in Fish, Chap. 21 in Fish Oils, Their Chemistry, Technology, Stability, Nutritional Properties and Uses, M. E. Stansby, Ed., Avi Publishing Co. (1967).

R. A. Stein, Vida Slawson, and J. F. Mead. Gas-Liquid Chromatography of Fatty Acids and Derivatives, Chap. 9 in Chromatographic Analysis of Lipids, G. V. Marinetti, Ed., Marcel Dekker (1967).

I. Harary, L. E. Gerschenson, D. F. Haggerty, Jr., W. Desmond, and J. F. Mead. Fatty Acid Metabolism and Function in Cultured Heart and HeLa Cells, in Lipid Metabolism in Tissue Culture Cells, G. H. Rothblat and D. Krichevsky, Eds., Wistar Press (1967).

Dorothy L. Fillerup and J. F. Mead. The Lipids of the Aging Human Brain. Lipids 2, 395 (1967).

Mona E. Fewster, A. B. Scheibel, and J. F. Mead. The Preparation of Isolated Glial Cells From Rat and Bovine White Matter. Brain Research 6, 401 (1967).

A. J. Fulco. Chain Elongation, 2-Hydroxylation and Decarboxylation of Long Chain Fatty Acids by Yeast. J. Biol. Chem. 242, 3608 (1967).

A. J. Fulco. Unsaturated Fatty Acids, The Encyclopedia of Biochemistry, R. J. Williams and E. M. Lansford, Jr., Eds., Reinhold Publishers (1967).

A. J. Fulco. The Effect of Temperature on the Formation of Δ^5-Unsaturated Fatty Acids by Bacilli. Biochim. Biophys. Acta 144, 701 (1967).

12. SCOPE OF THE PROJECT

Although there is incomplete 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 amplifies the effect of the radiation. Moreover, their importance in cellular membranes and the possibility that in their ordered arrangement in such membranes, the lipids would be most readily attacked and that the results of such attack might be fatal to the cell, necessitate studies of their radiation chemistry. There is thus a need to study the primary effect of ionizing radiation on the lipids and related substances both from the point of view of the fundamental nature of the changes involved and from that of their occurrence in living organisms. The proposed studies are to investigate the alterations in unsaturated fatty acids and other lipids with irradiation both *in vitro* and *in vivo* to assess the contribution of these reactions in the overall effect of irradiation on living organisms.

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

13. RELATIONSHIP TO OTHER PROJECTS

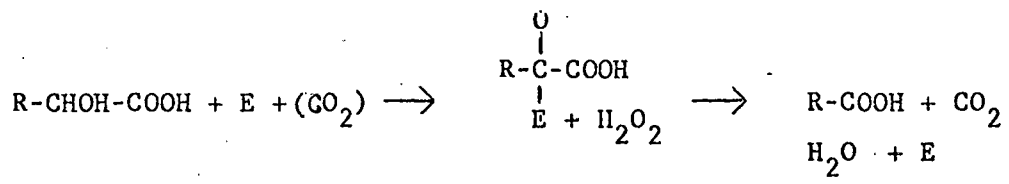
There are many projects throughout the country dealing with lipid chemistry and metabolism and the relationships of these to ionizing radiation. The principle investigators on these projects are generally well-known to each other and their research results undoubtedly influence the thinking in all organizations dealing with the subject, including this one. With the present state of communications, it is probably safe to say that important results become widely known even before publication.

14. TECHNICAL PROGRESS IN 1968

Our previous studies demonstrated that, with the exception of the gangliosides, the human brain lipids appear to change as a group with aging rather than individually, probably indicating a loss of myelin and its component lipids. Those fatty acids that could be derived from the diet, however, varied so widely among individuals as to mask any age-related changes. One class of fatty acids, however, must be entirely synthesized within the brain and might be expected to reflect any changes in the synthetic enzymes. These very long chain acids of the brain sphingolipids have been shown to increase in the older brains. Too little data are yet available, however, to ascertain whether this is a steady increase or whether it may occur largely in the early or late phases of aging.

Further studies have shown that human myelin ^{14}C content is approximately the same as that in atmospheric CO_2. This finding, which is contrary to all previous evidence, will require considerable confirmation before acceptance. Such studies have been initiated.

The nature of the alpha-oxidation system of the brain microsomes has become somewhat clearer. It now appears to be a series of oxidations requiring only oxygen and ferrous ion. Previous findings of a requirement for a reducing agent may stem from the necessity for keeping the iron in the reduced state. The nucleotide requirement may have been for a chelating agent to keep ferric iron in solution. It seems probable that the reaction from alpha hydroxy acid to unsubstituted acid plus CO_2 closely resembles that of lactic oxidative decarboxylase in that the intermediate keto acid is not released but is further oxidized by the other product of the initial oxidation-hydrogen peroxide.



In further studies on the effect of temperature on the nature of the membrane fatty acids, in vitro studies using homogenates from fish tissues have shown that ^{14}C-acetate is generally incorporated at a relatively higher rate into unsaturated fatty acids as the temperature of incubation is decreased. It has also been shown that white muscle from fish is incapable of activating fatty acids although the acyl CoA synthetase from dark muscle is comparable in this respect to that from the rat. In a series of microorganisms, it was found that those that produced Δ^5-desaturation were under strong influence of temperature, whereas those that desaturated in other positions (8,9,10) were not. In this case, the effect of the higher temperature may be in denaturation of the desaturating enzyme. The mechanism of the temperature effect in higher organisms is unknown.

15. EXPECTED RESULTS IN FY 1969

During this year it may be possible to compare the effects of aging and ionizing radiation on the long-chain fatty acids of the brain sphingolipids in a group of very old rats made available in another laboratory.

The alpha oxidation system will be examined further from the point of view of mechanism and the question of why its products appear to accumulate with age. The possibility that myelin itself contains enzymes effecting the turnover of brain lipids will be examined.

The mechanism of the temperature effect on desaturation reactions will be studied in microorganisms and the effect of temperature on fatty acid activation in dark muscle of fish will be examined.

Further work will be carried out on the metabolism of polyunsaturated fatty acids by cells in culture in an attempt to explain the loss of desaturating ability of the HeLa cell.

16. EXPECTED RESULTS IN FY 1970

Future research will naturally depend on the results obtained from current efforts. Nevertheless, it can be anticipated that by the end of the fiscal 1970, a good understanding of the effect of aging on the human brain lipids will have been achieved. The mechanism of α-oxidation and the many influences on it, such as aging, will be better understood. The study of the function of lipids in membranes and the relationship of structure and function will have been further clarified.

The function and metabolism of the polyunsaturated fatty acids in normal and cancer cells in culture will be further elucidated and the effect of irradiation on these parameters will be investigated.

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.:	4. Date Prepared:
06-04-00	April - 1968
5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA
7. Person in Charge:	8. Project Term:
David R. Howton	From: 1959 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2	2	2
(b) Other Tech.	½	½	½
Total	2 ½	2 ½	2 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 36,900	$ 39,400	$ 39,800
(b) Materials & Services	5,300	5,100	5,100
(c) Indirect Expenses *	(3%) 29,900	(3%) 30,200	(2%) 21,700
Total	$ 72,100	$ 74,700	$ 66,600

* 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 1968

D. R. Howton and Guey-Shuang Wu. Photolysis of α-Iodostearate. Abstracts, Joint Meeting, American Oil Chemists' Society and American Association of Cereal Chemists, Washington, D.C., April, 1968.

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 contact with aqueous phases, both direct and indirect effects are of immediate interest. Inasmuch as the usually unbranched saturated or cis-unsaturated hydrocarbon chains they contain are largely responsible for the characteristic physical and chemical properties of the lipids (and must also be intimately involved in the physiological function of these substances), related substances containing such groups serve as models for the envisaged studies.

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

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

13. RELATIONSHIP TO OTHER PROJECTS

Los Alamos Scientific Laboratory (H. W. Langham): "Behavior of Cell Membrane Mechanisms During and Following Gamma Radiation"; Studies at the Unilever Research Laboratory (England) under direction of Dr. Dennis Chapman (Head, Molecular Biophysics Unit); Pioneering Research Divn., U.S. Army Natick Laboratories (C. Merritt, Jr., et al.); Faculty of Pharmaceutical Sciences, Kumamoto Univ., Japan (K. Kitahara et al.) and at the Institute of Physical and Chemical Research, Bunkyo-ku, Tokyo (E. Fukada, et al.).

14. TECHNICAL PROGRESS IN FY 1968

Yields of hydrocarbon produced by exposure of saturated fatty acids, $CH_3(CH_2)_nCOOH$, where n = 10, 12, 14, 16, 18, and 28 to about 25 Mrads of Co^{60} γ-radiation have been determined by silicic-acid-column and gas-chromatographic techniques and found to vary inversely with chain-length, from 4.42 (for lauric) to 1.87 (for melissic acid); contrary to an earlier

claim of Whitehead et al. (J. Chem. Phys., 48, 184 (1951)), behavior of the latter in this regard is not anomolous. These data are being interpreted in terms of various possible modes of referal of initially-imparted excitation energy to the carboxyl group.

γ-Radiolysis of saturated fatty acids (on the basis of studies on stearic acid) leads to extensive decarboxylation but also, quantitatively comparably, to formation of polymeric material which appears to be initially predominantly dimeric and, on the basis of current concepts of mechanisms involved, presumably cross-linked at carbon atoms adjacent to the carboxyl group. To substantiate these tentative conclusions, it is highly desirable to know the silicic-acid-column-chromatographic behavior of authentic stearic acid dimer (α,α') (particularly as the dimethyl ester) and of the corresponding anhydride and a variety of details of the chemistry of interconversion of the dimer and its derivatives. Although such substances have been obtained by pyrolysis of dibutyl peroxide in the presence of methyl stearate (a reaction which may well be closely similar mechanistically to the radiolytic dimerization) and by more conventional approaches, the ostensibly simple peroxide synthesis is complicated by subsequent further reaction of the derived product to yield higher polymers. A new approach to the preparation of such substances has been explored: photolysis of α-iodo and -bromo esters and acids, expected to yield dimers and molecular iodine (or bromine) following homolysis of the C-X bond. Contrary to expectation, stearate and 2-octadecenoate are produced in important quantity. Although these are products expected to arise by disproportionative encounter of the intermediate α-carboxylate radicals, this is not expected to be an extensive eventuality with this type of radical and is contraindicated (as the mechanism of their formation) by the facts that the yield of the unsaturated product is initially greater than that of its saturated analog and that no octadecenoate is formed either in the dibutyl peroxide pyrolysis or γ-radiolysis reactions, both of which involve the same sort of α-carboxylate radicals. By analogy with studies of ethyl iodide photolysis by Hamill (JACS 78, 6228 (1956)), the olefinic product is believed to arise by hydrogen-atom abstraction by iodine atom before the latter escapes the cage embracing the homolysis fragments, and the stearate by abstraction of hydrogen from the HI thus produced. In accord with these views, methyl α-iodostearate yields 4% of the desired dimer (at 75% conversion of starting material exposed neat in a quartz vessel to low-pressure mercury arc illumination) while 25% dimer is obtained by use of the corresponding α-bromo ester, presumably because the more fugitive bromine atom has a higher probability of escaping from the cage. Both dl- and meso forms of the dimer are produced and these are identical (by the criterion of gas chromatographic behavior) with diazomethane-treated products of the γ-radiolysis of stearic acid.

Other studies have presented a need for 9-pentadecynoic acid (in which the acetylenic unsaturation is seven methylene groups removed from the carboxyl group, as it is in stearolic acid, the triple-bonded analog of oleic acid) and for isomeric substances in which the triple-bond-carboxylic-groups separation is (instead of seven) either six or eight methylene groups. Although the Ahmad-Strong synthesis (JACS 70, 3391 (1948)) makes any straight-chain acetylenic carboxylic acid potentially available, few have yet been prepared, aside from those (including stearolic) in the C_{18} series (see Huber, JACS 73, 2730 (1951)). Application of this general method to the problems at hand has resulted in a number of technical improvements, principal among which is use of dimethyl sulfoxide as solvent

in conversion of intermediate acetylenic chlorides to the corresponding nitriles (which are then hydrolyzed to the desired acids); this step, requiring about 4 days when aqueous ethanol is employed, can now be completed in a matter of minutes. Interestingly, the melting points of the pentadecynoic acids alternate strongly with position of the triple bond: 8-, m.p. 22°; 9-, m.p. 34°; and 10-, m.p. 25°. This behavior is in striking contrast with the 18-carbon homologs prepared by Huber (loc. cit.), which show surprisingly little m.p. variation, the 8-, 9-, 10-, 11-, and 12-octadecynoic acids all melting within the range 45.5 - 47.6°, and the 7-isomer only slightly higher (48.5 - 49.5°).

15. EXPECTED RESULTS IN FY 1969

Currently anticipated are studies designed to test a mechanism proposed (JACS 89, 516 (1967)) for γ-radiolytogenic decarboxylation of carboxylic acids, to determine yields of various products obtained by γ-radiolysis of oleic acid at low doses, and to establish the relative quantitative importance of several possible routes to polymerization of oleic acid on exposure to γ-rays. Efforts will also be directed toward interpretation of the dependence of decarboxylation yield on chain length of saturated fatty acids in terms of the mechanism of referral of primary radiolytic attack in the hydrocarbon chain to the carboxyl group.

16. EXPECTED RESULTS IN FY 1970

At such time as current studies of the mechanism of γ-radiolysis of free fatty (carboxylic) acids have progressed to a suitable point, attention will be directed toward lipids of progressively greater complexity — methyl esters, triglycerides, and phospholipids, with the ultimate objective of understanding the effects of ionizing radiation on the structure and function of the lipids of biological membranes.

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 - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Judd C. Nevenzel

8. Project Term:
From: 1962 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	1	2	2
(b) Other Tech.	½	½	½
Total	1 ½	2 ½	2 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 22,800	$ 33,800	$ 34,300
(b) Materials & Services	4,000	2,700	3,200
(c) Indirect Expenses *	(2%) 19,900	(2%) 20,100	(2%) 21,600
Total	$ 46,700	$ 56,600	$ 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 1968

None

12. SCOPE OF THE PROJECT

The Tracer Synthesis Section was established to prepare isotopically labelled molecules of interest in the biological or chemical investigations of the Biochemistry Division. To date various naturally-occurring fatty acids have been labelled with carbon-14 or tritium by techniques developed in this Division.

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

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

13. RELATIONSHIP TO OTHER PROJECTS

In the general areas of synthesis 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 deBiologie Vetetale, Bellvue, France, (P. Mazliak),

and Agriculture Research Service, USDA Beltsville, Md. (W. A. Gentner), Department of Biochemistry, the Connecticut Agricultural Experiment Station, New Haven, Conn. (P. E. Kolattukudy). Wax ester biosynthesis in marine animals is under investigation at the Hormel Institute, Austin, Minn. (H. Schlenk) and Hiroshima University, Japan (M. Kayama).

14. TECHNICAL PROGRESS IN FY 1968

Work continued on the lipid samples obtained during the 1966-67 cruises of the R/V Swan out of Santa Barbara, with attention focused first on the fate of 1-^{14}C-hexadecanol injected intramuscularly into lantern fish. 1.2 - 5.2% of the injected radioactivity was incorporated into the wax ester fractions of the lipids. On hydrolysis of the wax esters the derived alcohols were found to have 8-30 times the specific activities of the fatty acids. Even so, the specific activities of the latter were sufficient to permit the use of unlabelled carrier during their fractionation according to the degree of unsaturation, and subsequent separation of pure palmitic acid by reversed-phase column chromatography of the total saturated acids. By Schmidt degradation 94% of the carbon-14 of this palmitic acid was found to be in the carboxyl group, thus establishing that the direct oxidation of administered 16:0 alcohol to carboxylic acid does occur.

Calanoid copepods (Class Crustacea) from the Swan cruises have enabled us to confirm Lovern's original identification of long-chain alcohols from this source, made 30 years ago, and his recent postulate that wax esters should be important constituents of these lipids. Mixed copepods from the Santa Barbara channel contained major amounts of wax esters; those collected down to 800 m over Rodriquez Dome, and rich in the deep sea species, Gaussia princeps, yielded lipids containing 73% wax esters and only 9% triglycerides.

The technique of labelling polyunsaturated fatty acids with tritium by Wilzbach-exposure of derivatives in which the double bond system had been protected by bromination was judged a success. From 475 mg of irradiated methyl hexabromeicosanoate fractional crystallization of the free acid to constant specific activity finally gave 254 mg of 5,6,11,12,14,15-hexabromeicosanoic acid of 9.6 μCi/mg specific activity. Zinc debromination of a small aliquot and remethylation produced a sample of methyl eicosatrienoate of 17.3 μCi/mg specific activity, showing a single radioactive peak in gas liquid chromatography.

15. EXPECTED RESULTS IN FY 1969

The investigation of the pathways of carbon in the biosynthesis of the wax esters in fish will continue, with primary attention to working up the remaining samples from the R/V Swan cruises. Still to be examined in detail is the fate of injected palmitic and oleic acids. We will attempt to compare the pathways of ^{14}C-hexadecanol (a) injected *in vivo* and (b) incubated *in vitro* with tissue homogenates of muscle or liver. Additional *in vivo* experiments must await the availability of space on a research vessel equipped to permit sophisticated, on-board biochemical techniques. We would like to study the enzymatic reactions in cell-free systems, beginning with the lipase (or esterase) involved in the hydrolysis (and possibly the synthesis) of the wax esters in muscle.

16. EXPECTED RESULTS IN FY 1970

The direction the study of non-glyceride lipids in fish will take depends on progress in FY 1969. It is desirable to know more about the physical properties of the wax esters in comparison to triglycerides as affected by pressure in order to suggest possible function for the large amounts of the former present in the muscles of some deep-sea fishes. By histochemical techniques we hope to ascertain the location of the wax esters in lantern fish muscle as compared to that of triglycerides in other species.

The biosynthesis of wax esters, long-chain aldehydes, and alcohols in plant leaf waxes will be studied. The changes in the leaf wax and latex enzymes of Ficus species with age will be explored. Our isolated, rooted leaves of F. elastica are now four years old.

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 Separation Methods

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

4. Date Prepared: April - 1968

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

6. Working Location: UCLA

7. Person in Charge: Alexander Kolin

8. Project Term: From: 1967 To: 1968

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	1	0	0
(b) Other Tech.	-	-	-
Total	1	0	0

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 8,600	$ -0-	$ -0-
(b) Materials & Services	6,200	-0-	-0-
(c) Indirect Expenses *	(1%) 9,900	-0-	-0-
Total	$ 24,700	$ -0-	$ -0-

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

11. PUBLICATIONS DURING FY 1968

An Electromagnetic Catheter Flowmeter, A. Kolin, G. Ross and J. Archer, Clinical Research 16, 110, Jan. 1968

Helical Path Particle Electrophoresis in Endless Fluid Belts, A. Kolin, Biophysical Society, Feb. 1968

Continuous Flow Electrophoretic Separation and Characterization of Strains of Bacteria and Viruses, S. J. Luner and A. Kolin, Biophysical Society, 1968.

Electrophoresis in Endless Fluid Belts, A. Kolin, American Chemical Society, 1968.

An Electromagnetic Catheter-Flowmeter, A. Kolin, J. Archer and G. Ross, Circulation Research 21: 889-899, Dec. 1967

An Electromagnetic Catheter Flow Meter for Determination of Blood Flow in Major Arteries, A. Kolin, G. Ross, J. H. Grollman and J. Archer, Proc. Nat. Acad. Sci., (in press)

Ultraviolet Photography for Visualization of Separation Patterns of Zone Electrophoresis and Other Methods, Stephen J. Luner, Analytical Biochem. (in press).

12. SCOPE OF THE PROJECT

The primary concern of this project is to conceive new principles of particle separation. The particles of concern to this project range from medium size molecules to biological cells. Subcellular fragments are included in the objectives of this project.

New principles will involve new force effects which have, as yet, not found application in this field of endeavor as well as new methods of elimination of disturbing factors such as thermal convection. New physical properties are to be used whenever possible as a basis of separation, such as, for instance, the electrical conductivity and cell membrane permeability which will be utilized in separations based on electromagnetophoresis. Special emphasis will be placed on exploitation of the effect of electromagnetophoresis taking advantage of the available superconducting magnet. Forces exerted upon particles in heterogeneous magnetic fields are also to be explored.

One of the aspects of this work is concerned with separation and individual accumulation of different components of mixtures of biological particles. Another important objective is concerned with measurement of physical properties such as electrophoretic mobility, isoelectric point, etc. for the purpose of characterizing biological particles by precise measurements to facilitate their identification.

13. RELATIONSHIP TO OTHER PROJECTS

One of the most frequently encountered operations in the course of biological, biochemical, and biophysical research is separation of

molecular, subcellular, and cellular components of living systems from each other. The aim of this project is to evolve effective methods for the accomplishment of this aim and, hence, is intended to promote, through methodological developments, a great variety of projects carried on in this Laboratory of Nuclear Medicine and Radiation Biology as well as in other laboratories engaged in similar work.

14. TECHNICAL PROGRESS IN FY 1968

The method of endless fluid belt electrophoresis has been essentially worked out to completion and described in publications. In this method a fluid sheath resembling the shape of a belt, in a vertically mounted belt sender, is maintained in circulation by electromagnetic forces while a transverse electric field permits simultaneous electrophoresis. A fine jet, containing particles and ions to be separated, is injected and wound around the inner iron core in the shape of a non-circular helix. The separated components move along helices of different pitch and can be separately collected. This method has been applied to separation of small molecules, proteins, tissue culture cells, blood cells, yeasts, bacteria and viruses.

Concurrently, methods of blood flow determination by means of electromagnetic catheters were developed. It is anticipated that such methods will make it possible to check and standardize circulatory methods based on uses of radioactive isotopes. One type of electromagnetic catheter device measures blood flow through branch arteries of the aorta supplying various organs. A second type measures the blood flow through the main arterial trunk.

15. EXPECTED RESULTS IN FY 1969

This particular project was initiated on January 1, 1967 and was terminated on December 31, 1967.

16. EXPECTED RESULTS IN FY 1970

Same as 15.

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

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

4. Date Prepared:
April - 1968

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 1968	FY 1969	FY 1970
(a) Scientific	4 ½	4 ½	5 ½
(b) Other Tech.	¾	½	½
Total	5 ¼	5	6

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 60,500	$ 59,000	$ 72,900
(b) Materials & Services	6,400	10,200	12,700
(c) Indirect Expenses *	(4%) 39,800	(4%) 40,300	(5%) 54,000
Total	$ 106,700	$ 109,500	$ 139,600

* 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 1968

Ingalls, R.B., Myers, Jr., L.S., Holmes, D.E., and Glass, J.W.: An ESR Study of Reactions of 1,3-Dideuterouracil and 1,3-Dideutero, 5-Bromouracil with Hydrogen Atoms, Radiation Research, 31, 545-546, 1967.

Theard, L.M., Peterson, F.C., Willson, R.L., Ward, J.F., Myers, Jr., L.S., and Ingalls, R.B.: A High Resolution Pulse Radiolysis Apparatus - Some Initial Studies with Systems of Biological Interest, Radiation Research, 31, 581-582, 1967.

Holmes, D.E., Ingalls, R.B., and Myers, Jr., L.S.: An ESR Study of Free Radicals Formed by Reaction of Nucleotides and Their Constituents with Hydrogen Atoms, International J. Radiation Biology, 12, 415-426, 1967.

Holmes, D.E., Ingalls, R.B., and Myers, Jr., L.S.: ESR Spectra of Free Radicals Induced in Nucleic Acid Purine and Pyrimidine Bases and Selected Analogs by Exposure to Hydrogen Atoms, International J. Radiation Biology, (In Press).

Myers, Jr., L.S., Hollis, Mary Lynn, Ward, J.F., Willson, R.L., Holmes, D.E., and Ingalls, R.B.: Effects of γ-Rays, H$\cdot$ Atoms, and OH$\cdot$ Free Radicals on Nucleic Acids and Their Constituents, Polymer Preprints (In Press).

12. SCOPE OF THE 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 closely related to investigations in this Laboratory under J. F.Ward, R.L. Lehman, D.R. Howton, and E.H. Strickland, and generally so to those under O.A. Schjeide, J.F. Mead, and others. Many other Laboratories throughout the world are conducting studies in radiation chemistry and biochemistry. Those most closely related are:

J. Weiss and G. Scholes, University of Newcastle-upon-Tyne, England; B. Ekert, Paris, France; Warren Garrison, University of California, Berkeley; Cyril Ponnamperuma, Ames Research Laboratories, Moffet Field, Palo Alto, California; Peter Riesz, National Institutes of Health, Bethesda, Maryland: Richard Holroyd, Atomics International, Canoga Park, California; A. Mueller, Karlsruhe, Germany; R. Shulman, Bell Telephone Laboratories; W. Gordy, Duke University, Durham, North Carolina; Leslie Theard, Gulf General Atomic, Inc.,

San Diego, California, and R.B. Ingalls, Edgerton, Germeshausen, and Grier, Inc., Goleta, California.

14. TECHNICAL PROGRESS IN FY 1968

Studies of the mechanisms by which ionizing radiation causes changes in DNA and DNA constituents have been continued. Substantial progress has been made as follows:

Development of Improved Apparatus for Pulse Radiolysis: Members of this Section and of the Sub-Cellular Radiobiology Section (J.F. Ward, Section Head) have cooperated with Dr. Leslie M. Theard in the development of high speed pulse radiolysis equipment at Gulf General Atomic, Inc., San Diego, California. The equipment has the combined advantages of large radiation dose per pulse, excellent signal to noise ratio, and high light intensity for kinetic spectrophotometry. It can be used to study rates of radiation induced reactions which are completed within a microsecond, and by suitable adjustment, slower reactions as well. Specific applications are described below.

Pulse Radiolysis Studies of the Reactions of Radiation Produced OH· Free Radicals with DNA, Nucleic Acid Constituents, and Related Compounds: Rates of formation and decay, and absorption spectra of transients produced by reaction of OH· free radical with DNA, nucleic acid constituents, and related compounds have been determined. Dilute aqueous N_2O saturated solutions were exposed to 2-500 nsec 10 mev electron pulses. Radiation doses were 750-1700 rads per pulse. Conclusions were: (1) Decay of transients formed by reaction of OH· with thymine, uracil, deoxyribose, and thymidine is complex. More than one transient species is formed in nearly every system investigated. Parent-daughter relationships appear to occur in some cases. (2) Uracil and thymine transients follow different patterns in their decay, particularly in alkaline solutions. (3) Observations of the spectra of transient absorptions have confirmed the hypothesis of stationary state radiation chemistry that the site of attack by OH· free radical on thymine and 5-methylcytosine, but not on uracil, changes with pH. The pH at which the change occurs corresponds to the pK for ionization of the OH· free radical. (4) Similarly, observations on thymidine at pH 7 and 12.4 have confirmed that attack partially shifts from the base to the pentose as the pH is increased. (5) Comparison of spectra of thymine, thymidine, deoxyribose, and DNA suggests that under physiological pH conditions OH· attack on pentose part of the DNA double helix is a major reaction pathway. (6) The transient formed by reaction of OH· with DNA has been shown to have a long life time in comparison with speeds of chemical reactions, and to survive without observable changes in radical structure. These observations suggest the feasibility of chemical reversal of the action of OH· radicals provided that irreversible changes do not occur in less time than we can observe. (7) Preliminary studies have been made on the rates of formation of transient absorptions. These have given information on: a) comparison of rates obtained by direct observation of transient formation with rates obtained using the CSN^- competition method, b) effects of ionization of thymine and OH· on rates, c) direct comparison of rates of addition to thymine and abstraction from dihydrothymine to obtain a similar radical, d) some effects of pentose substituents on rates, and e) rate of reaction of OH· with DNA.

An ESR Study of Free Radicals Formed by Reaction of Nucleotides and Their Constituents with Hydrogen Atoms: Cytosine, pentoses, pentose phosphates, nucleosides, and nucleotides were suspended on quartz wool by freeze-drying and exposed to hydrogen atoms at room temperature inside an ESR cavity. Stable free radicals were observed in every case. Spectra of sugar radicals do not change shape during the course of the reaction. Those of cytosine radicals show a loss of resolution as the exposure progresses, suggesting that side reactions involving the amino group occur. The spectra of nucleoside and nucleotide radicals change in shape as the exposure progresses. The initial spectra resemble those of the constituent bases, whereas late spectra contain a large component of a sugar radical spectrum. Comparison of spectra induced by hydrogen atoms with those induced by deuterium atoms suggests that atoms add to the bases, abstract from the sugars, and bring about both types of reactions with the nucleosides and nucleotides. The nucleosides and nucleotides appear to form hydrogen atom addition products on the base moiety initially; and on further exposure to give radicals which resemble sugar radicals by a process depending in part on the presence of base radicals. Thus hydrogen atom attack on bases appears to be in effect transferred to the sugar. This process may be important in bringing about damage to the polymer backbone when nucleic acids are exposed to ionizing radiation.

ESR Spectra of Free Radicals Induced in Nucleic Acid Purine and Pyrimidine Bases and Selected Analogs by Exposure to Hydrogen Atoms: Purine and pyrimidine bases, selected to permit determination of effects of structure, were exposed to hydrogen or deuterium atoms within an ESR cavity. Spectra were recorded at intervals during the exposure. Conclusions about pyrimidines are: atoms add to all pyrimidines tested; addition is to the 5,6 double bond of 1,3-dideuterouracil; methyl groups on carbons 5 or 6 exert a dominant directive effect on addition; dihydropyrimidinyl radicals appear to have a near planar conformation at room temperature, but 1,3-dimethyl-dihydropyrimidinyl radicals are non-planar; steric blocking of H atom addition appears unimportant; and oxo and amino groups on carbon 4 have slightly different effects on the electronic structure of the radicals. Reaction of H atoms with 5,6 dihydropyrimidines gives the same radicals by abstraction as are formed by addition to the corresponding pyrimidine. All purines tested give radicals with nearly identical electronic structures.

An ESR Study of Reactions of OH· Free Radical with Simple Mercaptans and Aminothiols of Biological Interest: The initial reaction of cysteine and related sulfhydryl compounds with the OH· free radical in aqueous solution has been observed by ESR spectroscopy. The OH· radicals were generated by reduction of hydrogen peroxide with titanous chloride. These were mixed with a flowing solution of one of the sulfhydryl compounds just prior to its entry into an ESR cavity. Cysteine, cystine, cysteamine, and cystamine gave similar spectra, probably due to the $RCH_2S\cdot$ free radical. Pencillamine gave a different spectrum which probably is due to the $RC(CH_3)_2S\cdot$ radical. These results agree well with conclusions based on reaction mechanisms deduced from stationary state experiments. Successful competition of sulfhydryl compounds for the OH· radical has been observed in mixtures containing methanol. This result illustrates the possibility that the protective effects of sulfhydryl compounds in biological systems may be due to their action as scavengers of the OH· free radical.

Other Studies of Radiation Effects on Nucleic Acids and Their Constituents: Preliminary investigations have been made of radiation effects on DNA containing carbon-14 labeled thymine. The DNA was synthesized by Escherichia coli 15 TAU, extracted by the phenol method, and irradiated. One major and at least two minor labeled radiation products have been found. Attempts to identify these products are in progress.

Studies of the formation and decomposition of the thymine dimer by ionizing radiation have been continued. Stationary state radiolysis experiments have shown that the decomposition yields are extraordinarily dependent on conditions. Highest decomposition yields appear to be obtained when the hydrated electron is the attacking species. Pulse radiolysis and stationary state experiments have thus far not provided unequivocal evidence that excitation processes alone are responsible for dimer splitting in irradiated solutions.

Operation of the Radiation Facility: This Section has continued to operate the 10,000 curie cobalt-60 source and the 250 kvp x-ray unit for the entire laboratory.

15. EXPECTED RESULTS IN 1969

Investigations of the radiolysis of nucleic acids and their constituents will be continued. Emphasis will be on studies of the initial reactions of $OH\cdot$, $H\cdot$, and e^-_{aq} with these compounds. Pulse radiolysis will be used in attempts to determine the rates of the reactions, the sites of attack, the kinetic properties of the transient species formed, and the possibility of protection of nucleic acids by substances which compete for $OH\cdot$, etc. ESR will be used in attempts to identify products formed by $OH\cdot$ and $H\cdot$, and if techniques can be developed, $e^-_{aq}\cdot$. Attempts will also be made to identify and study the properties of organic radicals formed when nucleic acids absorb ionizing radiation directly. Stationary state studies of the radiolysis of carbon-14 labeled DNA will be continued, and the importance of excitation processes will be investigated. Emphasis on nucleic acids, as compared with nucleic acid constituents will be increased.

16. EXPECTED RESULTS IN FY 1970

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, and extension of the experiments to the virus and bacteria level of biological organization will probably be attempted. The particular problems to be investigated and possible consideration of other biologically important compounds will depend on the results of the preceding year's work. Increasing use will be made of ESR spectrometry and pulse radiolysis, and it is hoped that apparatus for measuring circular dichroism will be useful for investigating radiation induced changes in secondary structure of macromolecules.

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:
Sub-Cellular Radiobiology

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
John F. Ward

8. Project Term:
From: 1964 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	1	2	2
(b) Other Tech.	½	-	-
Total	1 ½	2	2

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 16,900	$ 27,000	$ 28,800
(b) Materials & Services	3,700	6,100	6,600
(c) Indirect Expenses *	(1%) 9,900	(2%) 20,100	(2%) 21,600
Total	$ 30,500	$ 53,200	$ 57,000

* 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 1968

Theard, L.M., Peterson, F.C. Willson, R.L., Ward, J.F., Myers, Jr., L.S., and Ingalls, R.B.: A High Resolution Pulse Radiolysis Apparatus - Some Initial Studies with Systems of Biological Interest, Radiation Research 31, 581, 1967.

Ward, J.F.: The Effects of Chloride Ions on the Radiation Induced Degradation of Pyrimidines in Aqueous Solution, Radiation Research 31, 651, 1967.

Ward, J.F. and Urist, M.M.: γ-Irradiation of Aqueous Solutions of Polynucleotides, International J. of Radiation Biology 12, 209-218, 1967.

Myers, Jr., L.S., Hollis, Mary Lynn, Ward, J.F., Willson, R.L., Holmes, D.E., and Ingalls, R.B.: Effects of γ-Rays, H$\cdot$ Atoms, and OH$\cdot$ Free Radicals on Nucleic Acids and Their Constituents, Polymer Preprints (In Press).

12. SCOPE OF THE PROJECT

This project encompasses various approaches to the assessment of damage to biological molecules produced by γ-irradiation both *in vivo* and *in vitro*. Particular attention is being focused on the genetic material - DNA and its constituents. It is the aim of the project to correlate chemically observed changes with effects measured in living cells.

13. RELATIONSHIP TO OTHER PROJECTS

This work is related to the investigations carried out under the direction of L.S. Myers, Jr., D.R. Howton and R.L. Lehman in this Laboratory. Studies related to this project are being carried out throughout the world. Most closely related are: J.J. Weiss and G. Scholes, University of Newcastle upon Tyne, England; A. Pihl, Norsk Hydro Institute for Cancer Research, Oslo, Norway; K.G. Zimmer, Institüt für Strahlenbiologie, Kernforschungzentrum, Karlsruhe, Germany; F. Hutchinson, Department of Molecular Biology and Biophysics, Yale University, New Haven Connecticut; W. Garrison and J. Holian, Lawrence Radiation Laboratory, Berkeley, California.

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

14. TECHNICAL PROGRESS IN FY 1968

γ-Irradiation of Aqueous Solutions of Polynucleotides: The radiation-induced degradation of aqueous oxygenated solutions of the polynucleotides of adenine, uracil, cytosine and hypoxanthine has been examined by chemical methods. Adjustment of pH and temperature enabled random coil structures to be compared with double helices. Measurements of the amounts of base destroyed by irradiation showed little difference between random coil structures and mononucleotides. In the double helical structure the base moieties are protected to varying extents, and this protection appears to be related to helix stability. The mixed double helix of poly-U-poly-A is exceptional in this respect. Both bases are more sensitive to radiation when involved together in this structure. Measurement of single-chain breaks showed no dependence on macromolecular structure and only slight

dependence on the base constituent of the polynucleotide. Hyperchromicity measurements of irradiated solutions showed a marked dependence of the double helix stability on the bases involved in the structure. The poly-A double helix is very stable, whereas the poly-U double helix breaks down readily.

Effect of Chloride Ion on the Radiation Induced Destruction of Pyrimidines, Purines, Nucleotides, and Nucleosides are Investigated: Previous work has shown that the extent of destruction of pyrimidines depends on pH, chloride concentration, pyrimidine concentration, and oxygen concentration. Abnormal destruction yields (7 times normal yields) occur only in pyrimidines with no substitutes in position 5 and 6, such as uracil and cytosine. Subsequent experiments with purines and nucleosides and nucleotides, (adenine, guanine; adenylic, cytidylic, guanylic, uridylic; uridine, cytidine, thymidine, adenosine, guanosine; deoxyadenylic, deoxycytidylic, deoxyguanylic and thymidylic) have shown a different effect. Except for thymidine and thymidylic acid, chloride ion shows a protective effect, i.e. yields for purine nucleoside and nucleotide destruction are decreased. The changes in yield show a pH dependence which closely follows that calculated for Cl_2^- formation. It seems that reaction of Cl_2^- with pyrimidine or purine is important in determining the extent of base destruction.

Radiation of DNA: Measurements of the radiation induced destruction of DNA at neutral pH have been carried out. The double helical DNA is irradiated at constant ionic strength in the presence and absence of chloride. The hyperchromicity of DNA in the absence of chloride is destroyed more readily than that in presence of chloride. The decrease in hyperchromicity is produced by base destruction, breakage of sugar phosphate backbone, and destruction of hydrogen bonding. Preliminary experiments to study the damage on the conformation of DNA in the presence and absence of chloride have been carried out using circular dichroism spectroscopy. Preliminary results suggest that the secondary structure of DNA in the absence of chloride is more readily attacked than that in presence of chloride.

Development of Improved Apparatus for Pulse Radiolysis: Members of this Section and of the Chemical Radiobiology Section (L.S. Myers, Jr., Section Head) have cooperated with Dr. Leslie M. Theard in the development of high speed pulse radiolysis equipment at Gulf General Atomic, Inc., San Diego, California. Details are given in the report of the Chemical Radiobiology Section.

Pulse Radiolysis of Aqueous Solutions of DNA Constituents in the Presence of Chloride Ion: The role of Cl^- ion in the radiolysis of DNA and its constituents is being studied by the pulse radiolysis technique. N_2O saturated solutions are used so that OH· free radical is the only significant reactive species produced by radiation. Under these conditions Cl^- is believed to react with OH· to form Cl_2^-: $OH\cdot + H^+ + Cl^- \rightarrow Cl\cdot + H_2O$ and $Cl\cdot + Cl^- \rightarrow Cl_2^-$. Cl_2^- has a strong absorption at 340-360 nm and hence its formation and disappearance can be monitored during and following a short radiation pulse by kinematic absorption spectroscopy. From such data rate constants can be determined for reactions of the type: $Cl_2^- + X \rightarrow$ products where X is a DNA or RNA constituent. Results have shown that ribose, deoxyribose, adenine, adenylic acid and deoxycytidylic acid are relatively unreactive with Cl_2^-. Other purine and pyrimidine bases, nucleosides, and nucleotides are reactive, however. Results have shown that the rate constants vary over a wider range than ·OH reaction rates for similar compounds.

Thus, it is possible for Cl_2^- to show a specificity of attack. Further experiments have been carried out to determine the nature of Cl_2^-. Reaction rates of Cl_2^- with ferrous ion were measured as a function of ionic strength, μ. The theoretical slope of a plot of the log of the rate constant against $\mu^{\frac{1}{2}}/(1 + \mu^{\frac{1}{2}})$ should be -2, but experimental results give a slope of -1.6. This shows that the identity of Cl_2^- is questionable.

Pulse Radiolysis of Polynucleotides: Pulse radiolysis and kinematic absorption spectroscopy have been used to determine the absorption spectra of transient species produced in the radiolysis of 0.01 M NaCl solutions of polynucleotides. Solutions were saturated with N_2O. Systems studied were: polyadenylic acid double helix, polyadenylic acid random coil, polyadenylic-polyuridylic acid complex in double helix form, polyuridylic acid random coil, and uracil. Results show that the transient observed is associated with the organic compound (it is not Cl_2^-), uracil and polyuridylic acid give different spectra, the poly U + A double helix gives a spectrum which is different from that of the poly A double helix, and poly A double helices and random coils give entirely different spectra. The base moieties appear to be partially protected by the double helix structure.

15. EXPECTED RESULTS IN FY 1969

Increasing use will be made of pulse radiolysis to test radiation mechanisms deduced from steady state work. Emphasis will be on radiation effects on genetic systems, and examination of possible reaction mechanisms leading to the observed effects. Circular dichroism will be used to study changes in conformation of DNA and polynucleotides on exposure to radiation. It is anticipated that emphasis on *in vivo* systems will be greatly increased.

16. EXPECTED RESULTS IN FY 1970

In vitro studies using pulse radiolysis, EPR, and circular dichroic spectrometry will be continued along lines suggested by the previous year's work. Extension of the studies to *in vivo* systems using bacteria and bacteriophage will be accelerated. Attempts will be made to correlate the results of chemically measured radiation induced changes with changes observed in bacterial 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 - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Ole Arne Schjeide

8. Project Term:
From: 1951 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3 ½	2 ½	3 ½
(b) Other Tech.	½	½	½
Total	4	3	4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 47,600	$ 39,200	$ 51,900
(b) Materials & Services	11,700	12,000	15,000
(c) Indirect Expenses *	(4%) 39,800	(3%) 30,200	(3%) 32,400
Total	$ 99,100	$ 81,400	$ 99,300

* 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 1968

Schjeide, O.A. and Lin, R. I-San: In Vitro Syntheses by Coated Vesicles (Synthesomes), J. Cell Biology 35, 121A, 1967 (abstract).

Schjeide, O.A.: Effects of Estrogens on Lipid Metabolism in the Chicken, Progr. Biochem. Pharmacol. 2, 268-275, 1967.

de Vellis, J. and Schjeide, O. A.: Effects of Estrogens, Irradiation, and Actinomycin D on Enzymes Concerned with Lipid Metabolism, Progr. Biochem. Pharmacol. 2, 276-282, 1967.

Schjeide, O. A., Lin, R. I-San, and de Vellis, J.: Molecular Composition of Myelin Synthesized Subsequent to Irradiation, Rad. Res. 33, 107-128, 1968.

Schjeide, O. A., Lin, R. I-San, and de Vellis, J.: Changes in Myelin Elaborated Following X-Irradiation, Rad. Res. 17, 1967 (abstract)

de Vellis, J., Schjeide, O. A., and Clemente, C.D.: Protein Synthesis and Enzymic Patterns in the Developing Brain Following Head X-Irradiation of Newborn Rats, J. Neurochemistry. 14, 499-511, 1967.

de Vellis, J. and Schjeide, O. A.: Time-Dependence of the Effect of X-Irradiation on the Formation of Glycerolphosphate Dehydrogenase and Other Dehydrogenases in the Developing Rat Brain, Biochem. J. (In Press), 1968.

12. SCOPE OF THE PROJECT

The primary objective of the Metabolic Radiobiology Section is to study the perturbations of intracellular systems by ionizing radiations, to determine how these changes are brought about and to relate the effects to the total organism. The systems that have received primary attention in this section include metabolic and organelle patterns of cells undergoing development or differentiation.

In preparation for - and in conjunction with - studies of effects of radiations in the cells of developing or differentiating organs, study will be directed toward normal parameters of certain phenomena - such as membrane synthesis - which are basic to the knowledge of biological systems in general. It is reasoned that such information should ultimately prove to be of value in the elucidation of radiation-induced effects and to the amelioration of such disturbances.

Specific Areas of Investigation:

1. Isolation and chemical analysis of developing myelin from different areas of the brains of head-irradiated neonatal rats.

2. DNA-RNA hybridization studies on: a) liver cells and oviducal cells forced into new differentiations by estrogens and progesterone respectively, b) embryonic liver cells which do not yield overt responses to estrogens, c) liver cells exposed to both estrogens and x-irradiation, d) areas of brain prior to and during myelination, e) areas of brain in hypophysectomized rats, f) areas of brain in rats exposed to x-irradiation.

3. Isolation and chemical and metabolic analysis of 3 important organelles from the avian oocyte. These include coated vesicles (which are especially numerous in small avian oocytes), transosomes (bags of ribosomes contributed by follicular cells surrounding the oocyte), and a rapidly labeling, high molecular weight RNA that is affixed to membranes within the cell and is probably a masked messenger RNA.

4. Study of parameters of membrane synthesis, especially the synthesis and apparent self-replication of elements of the plasma membrane (coated vesicles) and synthesis of endoplasmic reticulum as stimulated by estrogens in avian liver cells.

5. Study of passage of proteins into the cells and transport of proteins and lipids by coated vesicles.

13. RELATIONSHIP TO OTHER PROJECTS

This work is related to studies of radiation induced derangements of intracellular organelles, proteins and lipids which are being carried out on adult organisms in the Biochemistry Division of this Laboratory. Related investigations are also being carried out at Rochester, Argonne, Western Reserve, and Oak Ridge in the United States and in England, France, and Russia.

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

Collaborative studies are underway with: Dr. William Hahn, University of Washington; Dr. Lester M. Morrison, Loma Linda University; Dr. James Mead, U.C.L.A.; Dr. Jean de Vellis, U.C.L.A.; Mr. Edward Grellert, U.C.L.A.; Dr. Gary Lai, U.C.L.A.

14. TECHNICAL PROGRESS IN FY 1968

DNA and RNA of Coated Vesicles: Coated vesicles have been isolated from small oocytes (2-6 mm diameter) of laying hens by density gradient centrifugation. In some cases thymidine H^3 or uridine H^3 was injected intraperitoneally for 1 hour prior to removal of the eggs.

DNA plus RNA was salt extracted from isolated coated vesicles and centrifuged on a $CsCl_2$ density gradient for 72 hours. Although some DNA was resolved as a discrete band by this procedure, more of it was associated with lipoprotein and some of it was hybridized with RNA. Treatment with pronase for 5 days prior to salt extraction resulted in a larger discrete band of DNA of a density of circa 1.69. Total DNA in coated vesicles was found to be circa 0.3 per cent.

RNA plus DNA was extracted from coated vesicles by the hot (65°C) phenol technique following pre-treatment with sodium dodecyl sulfate. Most of the RNA was present as a band sedimenting at 3-10S. Two smaller bands sedimented at 16-18S and 26-28S respectively. Specific activity was highest in the slowest sedimenting band which appears to be largely membrane RNA. The two faster sedimenting peaks appear to represent ribosomal RNA. Total RNA in the coated vesicles is circa 4.0 per cent. Of this, approximately 60-70 percent is membrane RNA.

DNA-RNA Hybridization Studies on Estrogenized Livers: Total RNAs (uridine H^3 labeled and non-labeled) were extracted from livers of pullets exposed to estrogens for 2 hours and livers of normal pullets. Total uptake of uridine H^3 into RNA of estrogenized livers was circa 50 per cent greater than in control livers. Hybridization incubations were carried out at (68°C) for 18 hours with normal liver DNA present on nitrocellulose filter discs. RNA from estrogenized livers competed successfully for binding sites on the DNA with RNA from control livers. This indicates that treatment with estrogen does not turn off transcription of normally occurring RNAs. However, RNA from control livers was not able to compete completely with RNA from livers of estrogenized pullets. This indicates that treatment with estrogens results in the production of unique RNAs in the avian liver. No qualitative differences could be detected between RNAs from livers of pullets estrogenized for 2 hours and RNAs from livers of heavily-laying hens. This means that all of the RNAs required for production of yolk proteins by the liver are induced within the first 2 hours of exposure to estrogen and also that the liver of the laying hen is constantly producing unique RNAs.

RNAs from livers of estrogenized lizards (Uta stansburiana) compete with RNAs from livers of estrogenized pullets. This indicates conservatism in the genes responsible for yolk protein synthesis. However, RNAs from oviducts of estrogenized pullets (although unique as compared to RNAs from oviducts of control pullets) do not compete successfully with RNAs from livers of estrogenized birds for DNA binding sites. This is the first demonstration of organ specificity in RNA transcription as a result of exposure to the same hormone.

Carried out in collaboration with Dr. William Hahn, University of Washington.

DNA and RNA of Liver Cell Fractions: DNA (labeled with thymidine H^3) and RNA (labeled with uridine H^3 were extracted from nuclei, mitochondria, microsomes, microsomes minus ribosomes and supernatant of control and estrogenized avian liver cells. The method employed for extraction included pre-treatment for 2 minutes with sodium dodecyl sulfate at 60°C followed by hot (65°C) phenol. Membrane RNA (0-10S) was found to be labeled within 2 minutes of injection of roosters with uridine H^3 whereas a full half hour was required to significantly label ribosomal RNA. The sedimentation constants of membrane RNAs from nuclei and mitochondria were similar to those of the microsomal and supernatant fractions.

DNA was present in all membrane fractions and the supernatant and, of course, in the nuclei. However, the specific activities of the DNAs in the membrane and supernatant fractions were much higher than for the DNAs of the nucleus. It is tentatively concluded that at least a portion of the extra-nuclear DNA is not chromosomal DNA.

High Voltage Electrophoresis of Whole RNA from Control and Estrogenized Avian Livers: Studies of the electrophoretic mobilities of 0-10S RNAs and whole liver RNAs reveal striking differences in pattern. The 0-10S material from livers of control roosters migrates as three discrete bands at pH 3.5: one narrow band (#1) moving slowly toward the cathode, a broad band (#2), whose trailing edge never leaves the point of application, moving towards the anode and a narrower band (#3) moving relatively rapidly toward the anode. On the other hand, RNA extracted from the total homogenate consists primarily of a band which corresponds to band #2 of the 0-10S pattern with a much smaller amount of material being represented by a band corresponding to band #3 of the 0-10S pattern.

When estrogen is administered in vivo changes in pattern of the 0-10S material take place as a function of duration of treatment. The #1 peak disappears altogether within a day of exposure, beginning within 6 hours and the #3 peak broadens - i.e. more material is present as a trailing edge. This situation prevails up to at least 3 days of exposure to estrogen. However, in the case of birds that have been treated over a period of a week, a return toward the normal electrophoretic pattern is seen. Actinomycin D (0.1 μg/gram body weight) acts to return the pattern toward normal even in the presence of estrogen.

When the migration of RNAs is permitted to take place for a longer period than in the above cases, resolution of a new band (X) is observed on the leading edge of band #2 in RNA from livers of estrogenized birds.

From these data it would appear that band #2 contains nearly all of the RNA associated with ribosomes (but it probably also contains other types of RNA) since the bulk of the RNA in the whole homogenate is ribosomal RNA. The band moving most rapidly toward the anode would appear to be membrane RNA since its increase in amount upon exposure of the fowl to estrogen correlates with the increase in total membrane of the cytoplasm. (Upon exposure to estrogen the protein and lipid components of the microsomes increase more rapidly than does the microsomal RNA which is largely concentrated in the ribosomes.) The natures of the #1 band and the X band are not yet known and the migration characteristics of the transfer RNAs in this system also remain to be elucidated.

In Vitro Incorporation of Amino Acids by Coated Vesicles: Isolated coated vesicles from small avian oocytes were placed in an incubation medium similar to that employed by Seaman (1962) for study of protein synthesis by isolated kinetosomes from tetrahymena. No transfer RNA or pH 5 enzymes were added but the purine and pyrimidine triphosphates for synthesis of DNA and RNA were present. Radioactive amino acids were added at the beginning of the incubation. In the centrifugable coated vesicles radioactivity was present after 10 minutes of incubation but was much reduced at 20 minutes. Thereafter a steady increase in amount of label was observed up to approximaterly one hour after which time incorporation ceased. When 10% TCA was added to the total incubation mixture so that the total supernatant protein plus the intact coated vesicles could be centrifuged free of the incubation mixture, the activity in this combined fraction was found to rise smoothly, to show no drop at 20 minutes and to be higher overall than for the coated vesicles alone. The tentative interpretation for these phenomena is that coated vesicles incorporate amino acids into subunits forming mainly on their surfaces (surface subunits can be seen in electronmicrographs of coated vesicles sectioned in situ) and that naturally or during centrifugation the more fully formed subunits are disassociated from the surface (accounting for the loss of activity after 20 minutes of incubation). After further incubations there is some aggregation of released subunits so that they will centrifuge down without coagulation by TCA.

A Possible Messenger RNA in the Avian Oocyte: Electronmicroscopy reveals the presence of Balbiani Ring Granule-like entities passing through the nuclear pores and entering the cytoplasm. This material appears to associate with specific sites on membranes within the cytoplasm rendering these complexes so dense that they sediment through a 1.20 density layer during density gradient centrifugation. The RNA labels very rapidly and extensively

when uridine H^3 is given to the hen. It displays a sharp sedimentation constant of circa 40-50S and contains over 50 percent of uracil. It is not associated with ribosomes in the small eggs studied (2-6 mm diameter) and does not take up significant amounts of amino acids when incubated in suitable medium.

15. EXPECTED RESULTS IN FY 1969

DNA-RNA hybridization of (a) liver cells exposed to both estrogens and x-irradiation, (b) areas of brain prior to and during myelination, (c) areas of brain in hypophysectomized and hormone supplemented rats, (d) areas of brain in neonatal rats exposed to x-irradiation.

Correlation of syntheses of DNA, RNA, protein and lipid in coated vesicles and other membranous systems. Determination of whether or not subunits forming coatings of coated vesicles represent nascent membrane.

Information on effects of radiations on amounts of long chain and hydroxy fatty acids and plasmalogens in developing myelin.

Information on enzyme systems of coated vesicles and cytoplasmic supernatants of small (2-6 mm diameter) avian oocytes.

Identification of migrating bands of whole RNA during high voltage electrophoresis.

Detailed information on properties of membrane RNA and DNA in coated vesicles.

Electronmicroscopic and biochemical analyses of transport of yolk proteins across the plasma membrane and intracellular transport and destinations of these entities.

16. EXPECTED RESULTS IN FY 1970

Studies not completed in 1969 will be continued and appropriate problems in Developmental - and Radiation - Biology will be developed.

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:
Developmental Radiobiology

3. AEC Budget Activity No.:	4. Date Prepared:
06-04-00	April - 1968
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: Jean de Vellis	8. Project Term: From: 1964 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2	2 ½	2 ½
(b) Other Tech.	¾	½	½
Total	2 ¾	3	3

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 24,100	$ 28,100	$ 29,900
(b) Materials & Services	5,600	6,300	8,800
(c) Indirect Expenses *	(2%) 19,900	(2%) 20,100	(2%) 21,600
Total	$ 49,600	$ 54,500	$ 60,300

* 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 1968

de Vellis, J.: Effect of X-irradiation on Enzyme Formation and Nucleic Acid Synthesis and Degradation in the Developing Rat Brain, Biochemical J., 104, 39P, 1966.

de Vellis, J.: Glycerolphosphate Dehydrogenase Differentiation in the Developing Rat Brain: Effect of X-irradiation, Abstracts p. 208, First International Meeting of Neurochemistry, Strasbourg, France, July 1967.

Schjeide, O.A., I-san Lin, R. and de Vellis, J.: Molecular Composition of Myelin Synthesized Subsequent to Irradiation, Radiation Research, 33, 107-128, 1968.

de Vellis, J. and Schjeide, O.A.: Time-Dependence of the Effect of X-irradiation on the Formation of Glycerolphosphate Dehydrogenase and other Dehydrogenases in the Developing Rat Brain, Biochemical J.(In Press, 1968)

de Vellis, J. and Inglish, D.: Hormonal Control of Glycerolphosphate Dehydrogenase Activity in the Rat Brain, Fed. Proc. (In Press, 1968.)

12. SCOPE OF THE PROJECT

The purpose of the Developmental Radiobiology section is to study the effects of ionizing radiation on the biochemical differentiation of the developing rat brain. Parameters measured include specific types of DNA, RNA, lipids and regulatory enzymes. The goal of this work is to help elucidate the mode of action of ionizing radiation on biological systems and characterize the biochemical alterations induced by irradiation. Our previous studies have shown that irradiation alters the normal course of cell differentiation in the rat brain. For instance, the formation of several enzymes is inhibited and myelination of nerve axons is reduced. The alteration in enzyme patterns is permanent and there is no recovery toward a normal differentiated and functional state. Our previous results have indicated that the main biochemical lesion resulting form irradiation is perhaps at the transcription level. The differentiation process requires a controlled flow of the genetic information contained in the DNA of the cells and its implementation by the synthetic units of the cells. In order to fully understand the mode of action of ionizing radiation on brain maturation the mechanisms and factors regulating differentiation in brain cells should be known. To this end we have been studying the effects of hormones and inhibitors of protein and RNA syntheses on the level of key brain enzymes during brain development. Our studies have already partially elucidated (1) the sequence of biochemical alterations following irradiation (2) the relation of some enzymes to the functional differentiation of the brain and (3) the regulatory mechanisms involved in controlling the level of key brain enzymes.

13. RELATIONSHIP TO OTHER PROJECTS

This work is generally related to investigations in developmental and molecular biology under J. F. Mead, O. A. Schjeide and I. Harary in this Laboratory. Other laboratories conducting related studies include: (a) Radiation Neurobiochemistry: V. Nair, University of Chicago; P. S. Timiras, University of California, Berkeley; D. Ford and S. Cohan, S.U.N.Y., Brooklyn, N.Y. (b) In vivo effects of Irradiation on DNA and Enzyme Synthesis:

H. C. Pitot, University of Wisconsin; E. C. Pollard, Pennsylvania State University; K. C. Smith, Stanford University; H. Harrington, Western Reserve University; K. Dubois, University of Chicago; R. Haynes, University of California, Berkeley.

14. TECHNICAL PROGRESS IN FY 1968

Effect of X-irradiation on DNA During Brain Development: We reported previously that irradiation to the heads of rats at 2, 16, and 20 days of age causes brain DNA degradation and an inhibition of enzyme synthesis. Our tentative conclusion was that x-radiation causes reduction in the synthesis of some brain enzymes at the transcription level and that this process is probably related to DNA degradation. Additional evidence has been obtained to substantiate this concept. The loss of DNA from brain cells shows a biphasic dose-response curve, increasing rapidly until 375 r and very slowly at higher doses. This dose-response curve is very similar to that obtained for the inhibition of enzyme synthesis. However, the nuclear phosphorylation of histones whose inhibition by irradiation has been proposed to affect information transfer (Ord and Stocken, 1966) shows a biphasic dose-response curve similar to those mentioned above. Indeed, further information is needed to assess the possible role of DNA degradation and/or inhibition of phosphorylation of histones in the action of ionizing radiation on enzyme synthesis. Properties of the process of x-ray induced degradation of DNA was also investigated. Furthermore preliminary results indicate that the DNA of brain cells is not equally radiosensitive during the life cycle of the rat. At older ages less DNA is degraded.

Age-Dependence of the Effect of X-irradiation on Brain Enzymes: X-irradiation (100 to 1500 r) administered to the heads of rats from 8 to 30 days of age inhibited the development of glycerolphosphate dehydrogenase (L-glycerol-3-phosphate: NAD oxidoreductase, E.C. 1.1.1.8) in the brain stem and cerebral hemispheres. At 40 days of age and older no effect was observed. This inhibition was a delayed phenomenon, dose-dependent and with no recovery. It is proposed that the inhibition of enzyme formation is related to radiation damage caused to DNA. Actinomycin D inhibited the development of glycerolphosphate dehydrogenase in a manner similar to ionizing radiation. Four other dehydrogenases showed also age dependent radiosensitivities. Malic enzyme (E.C. 1.1.1.40) lactate dehydrogenase (E.C. 1.1.1.27) and malate dehydrogenase (E.C. 1.1.1.37) ceased to be radiosensitive at about 8 days of age and isocitrate dehydrogenase (E.C. 1.1.1.42) at 16 days. The correlation between developmental increase in enzyme activity and radiosensitivity holds closely for glycerolphosphate dehydrogenase and isocitrate dehydrogenase and to a smaller extent for the others.

Hormonal Control of Glycerolphosphate Dehydrogenase Level in the Rat Brain: Following hypophysectomy or adrenalectomy glycerolphosphate dehydrogenase (GPDH) (E.C. 1.1.1.8) activity decreased exponentially in the cerebral hemispheres and brain stem of adult male rats. The latter region was more affected than the former. But malate dehydrogenase (E.C. 1.1.1.40), isocitrate dehydrogenase (E.C. 1.1.1.42), lactate dehydrogenase (E.C. 1.1.1.27) and mitochondrial glycerolphosphate dehydrogenase (E.C. 1.1.95.5) activities remained unchanged. Injection of ACTH or cortisol in hypophysectomized rats or cortisol in adrenalectomized rats restored GPDH activity. Thyroidectomy and gonadectomy had no effect on GPDH activity. Liver GPDH

was not decreased by hypophysectomy or adrenalectomy. Muscle GPDH was diminished slightly by adrenalectomy and as much as brain GPDH by hypophysectomy. In young rats GPDH developmental increase in activity was inhibited by hypophysectomy. These results clearly show that brain GPDH activity is specifically regulated by cortisol (and probably closely related corticosteroids). This system is allowing us to study the mechanism of action of hormones on enzyme synthesis in the brain as well as to investigate the effect of ionizing radiation on the hormonal induction of GPDH enzyme in the developing and adult rat brain.

Book on Cell Differentiation: Dr. Schjeide and myself were fortunate to obtain the collaboration of 18 researchers prominent in the field of Developmental Biology to write an advanced textbook on "Cell Differentiation". Dr. Schjeide and myself are serving as editors and the book will be published by Van Nostrand Co. The book deals with the molecular and structural aspects of differentiation at the intracellular level.

15. EXPECTED RESULTS IN FY 1969

The research projects concerned with the effects of ionizing radiation on brain DNA and enzyme synthesis will be continued. The hypothesis that irradiation causes a reduction in genetic transcription (formation of mRNA) will be tested by DNA-RNA hybridization techniques. The radiosensitivity of DNA from various cell organelles will be investigated during development. Thus far we have not been able to detect any DNA repair in the brain following irradiation. Since this is an important point attempts will be made to clearly establish whether repair occurs or not.

The effect of ionizing radiation on the hormonal induction of brain glycerolphosphate dehydrogenase will be fully investigated in the adult and in the developing rat brain. This enzyme system offers us a rare opportunity since it is the only known brain enzyme which is under complete and specific hormonal control in the adult and developing brain.

16. EXPECTED RESULTS IN FY 1970

We anticipate continuing along the present line of research. The effect of ionizing radiation on the synthesis of a brain enzyme inducible in tissue culture will be studied if such a system can be established. Tissue culture will provide a better control of the environment and therefore a simpler system to study the mode of action of ionizing radiation on the synthesis of brain enzymes and differentiation in general.

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 - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Noel de Terra (Whittaker)

8. Project Term:
From: 1961 To: October 1967

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	½	0	0
(b) Other Tech.	¼	0	0
Total	¾	0	0

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 8,000	$ -0-	$ -0-
(b) Materials & Services	1,300	-0-	-0-
(c) Indirect Expenses *	(1%) 9,900	-0-	-0-
Total	$ 19,200	$ -0-	$ -0-

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

11. PUBLICATIONS DURING FY 1968

de Terra, N.: Macronuclear DNA Synthesis in Stentor: Regulation by a Cytoplasmic Initiator, Proc. Nat. Acad. Sci. U. S. 57, pp. 607-615, 1967.

12. SCOPE OF THE PROJECT

In this project a study is made 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 lead to a more complete understanding of important cell activities such as cytodifferentiation and provide valuable background information for studies of abnormal cell growth and function.

The regulatory mechanisms of unicellular organisms are investigated by experimental studies on cytoplasmic control of nuclear morphology and function in the ciliate Stentor.

Techniques include cell micrurgy (enucleation of cells, transplantation of nuclei), autoradiography and histochemistry.

13. RELATIONSHIP TO OTHER PROJECTS

Cell regulatory mechanisms are now the subject of intensive study in many laboratories around the world. The following workers are currently investigating the regulatory mechanisms of unicellular organisms: T. M. Sonneborn, University of Indiana, Bloomington; Jean Brachet, Universite Libre de Bruxelles, Belgium; O. H. Scherbaum, University of California, Los Angeles; Lester Goldstein, University of Pennsylvania, Philadelphia; Vance Tartar, University of Washington, Seattle; Konrad Keck, Johns Hopkins University, Baltimore; D. M. Prescott, University of Colorado, Boulder; J. B. Gurdon, Oxford University, England.

14. TECHNICAL PROGRESS IN FY 1968

Cytoplasmic Control of Macronuclear DNA Synthesis in the Ciliate Stentor coeruleus. III. Species Specificity of the Initiator: Transfer of Nuclear Nodes from Stentor polymorphus to Stentor coeruleus: The fact that nuclear nodes can be transferred into S. coeruleus from the slightly smaller species S. polymorphus makes it possible to determine whether or not the cytoplasm of one stentor species can support the occurrence of DNA synthesis within the nucleus of another species.

Large cells from a well-fed S. polymorphus culture were selected as donors and chains of nuclear nodes transferred from them into 18-24 hour-old S. coeruleus which were synthesizing DNA. In one experiment, the host cells were allowed to recover from the operation for 1 hour before a two-hour incubation in tritiated thymidine (100 μc/ml). In a second experiment, the recovery period was extended to 4 hours. Autoradiography of the host cells allowed to recover for 1 hour showed very

light label over the coeruleus nodes and no label over the polymorphus nodes. By contrast, the cells allowed to recover for 4 hours showed very heavy label over the coeruleus nodes and a light but definite label over the polymorphus nodes. The operation was apparently followed by a temporary inhibition of DNA synthesis or of thymidine uptake. However, the result of the experiment which employed a 4-hour recovery period shows that S. polymorphus nuclei transferred to S. coeruleus cytoplasm are capable of DNA synthesis if given time to recover from the immediate consequences of the operation. These findings suggest that the cytoplasmic factor which initiates and maintains DNA synthesis in Stentor is not species-specific.

15. EXPECTED RESULTS IN FY 1969

This activity has been discontinued because the Responsible Investigator left the Laboratory when her husband left the city.

16. EXPECTED RESULTS IN FY 1970

Same as 15 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:
Physical Radiobiology

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

4. Date Prepared: April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
E. Hardin Strickland

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3 ½	3 ½	4
(b) Other Tech.	½	½	½
Total	4	4	4 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 38,900	$ 41,400	$ 47,500
(b) Materials & Services	5,700	5,600	5,600
(c) Indirect Expenses *	(3%) 29,900	(3%) 30,200	(3%) 32,400
Total	$ 74,500	$ 77,200	$ 85,500

* 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 1968

Strickland, E.H.: Circular Dichroism of Horseradish Peroxidase and its Enzyme-Substrate Compounds, Biochim. Biophys. Acta 151, 70, 1968.

12. SCOPE OF PROJECT

This project focuses on elucidating the relationship between function and structure of macromolecules, both proteins and nucleic acids. Primarily we are investigating the conformations of enzymes and attempting to determine which aspects of conformation influence enzymatic activity. Circular dichroism has been used for our conformation studies since this is the most incisive technique presently available to determine conformation of enzymes in solution. Since studies on circular dichroism are still relatively new, much remains to be done to completely validate the interpretation of circular dichroism spectra. Present evidence suggests that ultraviolet circular dichroism gives information about helix, β-structure, and side chain orientations in proteins. One essential aspect of our project is to determine the underlying physical basis for the conformation-dependent circular dichroism.

Circular dichroism provides a unique tool for investigating the effects of ionizing radiation on the conformations of nucleic acids and enzymes. Interpretation of these circular dichroism spectra appear possible when appropriate preliminary experiments have been completed for compounds having known conformations.

13. RELATIONSHIP TO OTHER PROJECTS

The relationship between enzyme conformation and activity is studied by Dr. D.E. Koshland at Berkeley, Dr. Sherman Beychok at Columbia, Dr. W.F. Mommaerts at UCLA, and others. Dr. Norman Simmons (LNMRB) and Dr. Alex Glazer of UCLA, Dr. Elkan Blout at Harvard, and Dr. Dan Urry in Chicago have been active in studies relating to the interpretation of circular dichroism spectra.

The effects of ionizing radiation on proteins and nucleic acids have been investigated by numerous workers. There do not seem to have been any investigations using circular dichroism to study radiation damage, probably because these instruments are only now becoming generally available. Our radiation experiments are being closely coordinated with Dr. L.S. Myers, Jr., and Dr. J.F. Ward, LNMRB.

14. TECHNICAL PROGRESS IN FY 1968

Studies on horseradish peroxidase have been extended to the isoenzymes isolated by Drs. Shannon and Kay. These studies show that the two isoenzymes differing greatly in amino acid composition have nearly the same conformation. The native conformation is greatly stabilized by the heme moiety. Differences in amino acid content do, however, influence the stability of peroxidases, especially in the absence of heme.

The recent acquisition of a prototype of the Beckman far UV-CD spectrophotometer has increased our capability to measure circular dichroism spectra, especially in the far ultraviolet. By adapting a computer of

average transients to this instrument, we have obtained exceptional resolution of circular dichroism spectra in the near ultraviolet. These high resolution spectra have permitted tentative identification of the moieties responsible for circular dichroism bands between 260 and 290 mμ.

Preliminary studies were begun on nucleic acids.

15. EXPECTED RESULTS IN FY 1969

The factors responsible for the conformation of peroxidase isoenzymes will be investigated more extensively. The effects of Co^{60} gamma irradiation will also be examined. Studies will be initiated to examine possible allosteric transitions in enzymes showing feedback inhibition.

In an effort to obtain better resolution of circular dichroism bands in the near ultraviolet, measurements will be made at liquid nitrogen temperatures on proteins and amino acid derivatives frozen in glycerol-water glasses. Increased resolution of these bands would permit conclusive identification of the moieties involved in the near ultraviolet circular dichroism bands.

An extensive study will be made of the effects of Co^{60} gamma irradiation on the conformations of nucleic acids.

16. EXPECTED RESULTS IN FY 1970

Circular dichroism measurements on proteins and nucleic acids will be continued. A rapid response circular dichroism spectrophotometer will be developed for studies on DNA conformation changes during pulse radiolysis.

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 - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Hideo Nishita

8. Project Term:
From: 1959 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	4	4	4
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total	4 $\frac{1}{4}$	4 $\frac{1}{4}$	4 $\frac{1}{4}$

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 49,700	$ 51,300	$ 56,000
(b) Materials & Services	3,800	4,200	4,400
(c) Indirect Expenses *	(4%) 39,700	(4%) 40,300	(4%) 43,200
Total	$ 93,200	$ 95,800	$ 103,600

* 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 1968

Influence of Minerals on Sr90 and Cs137 Uptake by Bean Plants. Soil Science: In press. 1968.

Some Thermoluminescent Characteristics of Gamma Irradiated Soils. Soil Science: Accepted for publication. 1968.

12. SCOPE OF THE PROJECT

The general objectives of the Soil Factors Section are geared to one of the objectives of Environmental Radiation Division to study the biological cycling of radionuclides released into the environment as a result of utilization of nuclear energy. This Section is concerned with soil-plant interrelationship problems with particular emphasis on the chemistry of fission products and neutron induced radionuclides in soils. The objectives of this Section also include finding practical means of reducing the plant uptake of certain hazardous long-lived radionuclides, particularly Sr90, which is usually taken up in relatively large amounts by plants grown in contaminated soils. This Section is also concerned with the effects of ionizing radiation on soils.

Currently, this Section is involved in three projects:
(1) Influence of minerals on Sr90 and Cs137 uptake by bean plants grown on contaminated soils.
(2) Thermoluminescence of gamma-irradiated soils.
(3) Influence of heating of soils on the availability of Cs137, Sr90 and certain mineral nutrients to plants.

The scope and the objective of these projects are discussed below in the "Expected Results" section. These investigations are conducted in the laboratory and controlled plant growth facilities. Certain facets of the study of soil thermoluminescence will be done at the radiation field in Rock Valley, Nevada Test Site.

13. RELATIONSHIP TO OTHER PROJECTS

Research is correlated with other sections of the Environmental Radiation Division: Plant Factors, E. M. Romney (480311); Environmental Factors, H. A. Hawthorne (480320); Plant Physiological Ecology, A. Wallace (480344); Physiology of Mineral Accumulation, O. R. Lunt (480345).

Related studies at other laboratories:

U. S. Department of Agriculture, Beltsville, Maryland
Biology Department, Battelle-Northwest, Richland, Washington
University of California, Riverside, California
University of California, Berkeley, California
University of Tennessee, Knoxville, Tennessee
Ecology Section, Oak Ridge National Laboratory, Oak Ridge, Tennessee

14. TECHNICAL PROGRESS IN FY 1968

The influence of the addition of several minerals (clinoptilolite, "Uerxite," bentonite, illite, kaolinite, vermiculite and bauxite) on the

Sr90 and Cs137 uptake by bean plants grown in two contaminated soils was examined opposed to surface application. In these experiments the minerals were mixed into the soils. The Cs137 uptake by the plants was relatively small compared to Sr90 uptake with all mineral treatments. Among the minerals studied, clinoptilolite had the greatest effect on both the Sr90 and Cs137 uptake. The Sr90 uptake was significantly reduced, while the Cs137 uptake, although small, was increased. The effect of the other minerals ranged from no effect to small effects. Mineral treatment of soil either decreased the plant yield or had no effect.

Thermoluminescence of nine soils that were widely different in chemical and physical characteristics and certain minerals and salts that may occur in soils was examined. Soils irradiated to 1,000 R with Co60 gamma rays showed only small amounts of thermoluminescence, while others showed large amounts. The glow curves of some soils showed one broad peak, while those of others showed two or three broad peaks. The maximum peak heights generally occurred in one or several of the following temperature ranges---around 150° C and within 285° C to 295° C and 350° C to 375° C.

Certain minerals (vermiculite, kaolinite, bentonite, illite and bauxite) and salts (Fe_2O_3, $CaCl_2$, $CaSO_4$, Al_2O_3, K_3PO_4, CaO, $CaCO_3$, Na_2CO_3 and NaCl) that may occur in soils also showed wide ranges of thermoluminescence. In most cases, the maximum peak heights for these materials occurred in one or more of the temperature ranges listed above for the soils studied.

For a given soil, the shapes of the glow curves varied appreciably with gamma dose, but when the areas under the glow curves were plotted as a function of dose on logarithmic paper, sections of linearity were obtained. This indicated that the total energy absorbed was consistent with dose.

The plant growth phase in the study of heating a soil on the plant availability of certain elements was completed. The chemical analysis of the plant samples are in progress.

15. EXPECTED RESULTS IN FY 1969

(1) Influence of minerals on Sr90 and Cs137 uptake by bean plants grown on contaminated soils.

One of the problems that arises with the contamination of the biosphere with radioactive materials is finding means of contending with the dispersed radionuclides if they are at a biologically hazardous level. In order to minimize the transfer of hazardous radionuclides along the soil-plant-animal food chains leading to man, various methods that might be used on contaminated agricultural land have been studied.

The objective of this project is to determine the effect of application of different primary and secondary minerals on the Cs137 and Sr90 uptake by plants grown in contaminated soils. A summary of the results obtained thus far is given above under "Technical Progress for FY 1968." It is anticipated that this project will be terminated during this fiscal year.

(2) Thermoluminescence of gamma-irradiated soils.

The objectives of this project is to determine whether or not soil can be used as a radiation dosimeter. In order to do this, thermoluminescent characteristics of soils having widely different chemical and physical properties are under examination. A summary of the results obtained thus far is given above under "Technical Progress for FY 1968." It appears that by using certain procedures soils may be used as a radiation dosimeter. These procedures will be tested in the field (study site at Rock Valley, Nevada Test Site) during this fiscal year.

(3) Influence of heating of soils on the availability of Cs137, Sr90 and certain mineral nutrients to plants.

The soil in the vicinity of a nuclear detonation may be exposed to a wide variation of heat ranging from temperatures well above soil fusion down to ambient temperature with increasing distance from ground zero. The heat may change the plant availability of the radioactive, as well as the stable, nuclides in the soil. The objective of this project is to determine the changes in the availability of the various nuclides to plants as a function of soil heating. The plants from one experiment completed under this project is now being analyzed.

16. EXPECTED RESULTS IN FY 1970

Certain aspects of the projects in progress during FY 1969 will be continued. It is anticipated that a study on the effect of organic matter on the fixation of Cs137 in soils will be initiated.

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 - 1968

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

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Evan M. Romney

8. Project Term:
From: 1953 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	4	4	4
(b) Other Tech.	1/4	1/4	1/4
Total	4 1/4	4 1/4	4 1/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 53,100	$ 54,600	$ 58,200
(b) Materials & Services	4,700	5,300	5,300
(c) Indirect Expenses *	(4%) 39,700	(4%) 40,300	(4%) 43,200
Total	$ 97,500	$100,200	$106,700

* 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 1968

Behavior of Rare Earth Elements in Plants and Soils. Amer. Assoc. Adv. Sci. June 19-22 (Abstract) (1967).

Airborne Radioactivity in Fallout Areas. UCLA Report #12-628 (1967).

Redistribution of Plutonium at the Nevada Test Site. Health Physics (in press) (1968).

Vibration-Flow, A New Method of Pellet Washing. Amer. Ind. Hyg. Assoc. Jour. 28: 388-390. (1967).

Radiological Soil Analysis, Pacific 1952. UCLA Report #12-654. (1967).

12. SCOPE OF THE PROJECT

Research objectives of the Plant Factors Section are three-fold in scope: (1) improve understanding of plant functions in the biological cycling of radioactive isotopes deposited in desert environments, (2) further evaluate the susceptibility and response of desert plants to ionizing radiation from Plowshare nuclear testing events at the Nevada Test Site, (3) investigate the mineral nutritional characteristics of desert plants and their subsequent response to the manipulation of environmental factors known to influence plant growth. These objectives aim to better understanding of fallout irradiation damage to plants and also of fundamental mechanisms involved in plant uptake of radionuclides and mineral elements in the desert ecosystem.

Research is conducted in both field and controlled laboratory environments to help bridge the gap between practical and theoretical problems arising from radioactive fallout contamination. Results should help establish parameters for assessing the consequences to man of radioactive contamination in the environment and increase knowledge of the mineral nutritional characteristics and peculiarities of desert plants.

13. RELATION TO OTHER PROJECTS

Research is correlated with the other sections of the Environmental Radiation Division and collaborative studies are conducted with A. Wallace and O. R. Lunt. Related research is conducted at the following locations: Biology Department, Battelle-Northwest Laboratory; Radiation Ecology Section, Oak Ridge National Laboratory; Biology Department, Brookhaven National Laboratory; Biology Department, Emory University; University of California Lawrence Radiation Laboratory.

14. TECHNICAL PROGRESS IN FY 1968

We have continued to study biological cycling of radionuclides in areas of the Nevada Test Site contaminated by Plowshare testing events and in offsite areas contaminated with fallout from the 1955 and 1957 weapons testing series. Radiochemical analyses in progress indicate that considerable Sr^{90}, Ru^{106}, Cs^{137} and Ce^{144} is still cycling in plants, small rodents and jackrabbits ten years post-contamination. At sites where concentrations of neutron induced radionuclides are high there is evidence

of considerable cycling of radioactive Al, Co and Mn in plants and small mammals.

Among the most significant progress in Fy 1968 is the development of techniques to grow species of native desert plants under artificial laboratory conditions, thus permitting corroborative physiological and radiological studies in controlled environments in support of field investigations. Work now in progress includes studies on plant irradiation sensitivity and damage from a Co60 source, plant response to alteration of environmental factors, phenology and growth behavior, radionuclide uptake and fundamental mineral nutritional characteristics of desert plants.

A series of environmental study plots were established at the Nevada Test Site in cooperation with A. Wallace to help interpret effects of ionizing radiation on flora of the desert-type ecosystem. Native shrub species appear to be less sensitive to radiation damage than most cultivated plants. In two different fallout contaminated areas where no effects of blast or thermal damage occurred, the irradiation damage to vegetation was not accountable on the basis of gamma dosage alone; however, the additional beta dosage primarily from fallout particles adhering to the foliage caused severe damage that became evident within a few weeks post-irradiation. The combined effects of beta and gamma radiation must be taken into account in determining the sensitivity of plants to radioactive fallout contamination. Another system of test plots was established near a source of water at Mercury, Nevada and in Rock Valley to study factors which limit primary productivity in the desert ecosystem and also to determine some plant species interrelationships. Environmental manipulations being used at these plots include irrigation, nitrogen and phosphorus fertilization, metal chelate additions and insect control. These plots are being censused by a scheme that will permit computer analysis of forthcoming data. Sharp demarcation lines often exist where vegetation changes from one dominant species to another. Such ecotone lines involving Larrea divaricata and Atriplex canescens are being investigated in Frenchman Flat and another involving Artemisia tridentata, Erotia lanata and Juniperus osteosperma on Pahute Mesa. The causal factors involved at these ecotone lines appear to be soil texture and aeration, drainage and salt concentration.

A soil chemistry laboratory has been established at Mercury, Nevada and equipped to process soil samples from the environmental study plots and the soil survey of major areas of Nevada Test Site.

15. EXPECTED RESULTS IN FY 1969

We shall continue to investigate biological cycling of radionuclides in areas contaminated with fallout from Plowshare nuclear testing events. Completion of radiochemical analysis on soil, plant and animal samples from our persistence study areas is expected and our findings will be organized into reports for publication. Studies of irradiation effects on flora (recovery and succession) also will be continued to further evaluate the susceptibility and response of desert plants to radioactive fallout contamination. Work in the environmental study plots will continue to evaluate the effects of manipulating certain environmental factors on desert plant species. Mineral cycling studies will be intensified and they are expected to involve the use of some radioisotopes for diognostic purposes. We expect to complete installation of instruments for recording

soil moisture and temperature and also make considerable progress on the physical and chemical characterization of soils from the environmental study plots.

Investigations will be continued in our laboratory, glasshouse and controlled environment facilities to support and confirm findings from field studies with emphasis being placed on further evaluation of radiation sensitivity and basic physiology of desert plants. Considerable effort will be spent analyzing plant samples for mineral element contents using our newly developed optical emission spectroscopy techniques.

16. EXPECTED RESULTS DURING FY 1970

We expect to continue all phases of field and laboratory work in progress and also establish additional field study plots if further needs are indicated by research findings. Continued involvement with Plowshare nuclear testing events is desirable to the extent of helping reach our research project objectives. Computer analysis of data collected from environmental study plots in FY 1969 will be initiated and we expect to make considerable progress on the soil survey of major ecology study areas at the Nevada Test Site. Data from our mineral cycling studies may be used to help develop computer programs for a systems analysis approach toward understanding the desert 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:
Environmental Factors

3. AEC Budget Activity No.:	4. Date Prepared:
06-05-01	April - 1968
5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA, St. George, Utah, and Nevada Test Site
7. Person in Charge:	8. Project Term:
Howard A. Hawthorne	From: 1956 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	4 1/2	4	4
(b) Other Tech.	3/4	3/4	3/4
Total	5 1/4	4 3/4	4 3/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 51,100	$ 49,100	$ 52,700
(b) Materials & Services	8,100	8,500	8,500
(c) Indirect Expenses *	(4%) 39,700	(4%) 40,300	(3%) 32,400
Total	$ 98,900	$ 97,900	$ 93,600

* 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 1968

Effect of moisture tension on the concentration of tracer Sr^{85} in expressed soil solution. H. Nishita and H. A. Hawthorne. Soil Science 103: 339-346. 1967.

12. SCOPE OF THE PROJECT

From 1961 intensive studies were conducted, at field stations and in the laboratory, to obtain data from which stochastic models could be constructed; the models will summarize the effects that environmental factors had upon the biological cycling of fission products. Collection of materials from a dairy farm was emphasized from FY 1963 through FY 1966. Studies in FY 1967 were limited to definition of the growth curves for two cuttings of alfalfa plus two preliminary feeding trials, to assess the effect of high and low temperatures upon secretion of radionuclides into milk. Data reduction of the results of radiochemical analyses completed to date will require more than two years by the present staff.

13. RELATIONSHIP TO OTHER PROJECTS

Collaborative field studies are in progress with the Ecology of the Nevada Test Site Section. Laboratory studies of concurrent interest are conducted with the Soil Factors Section.

14. TECHNICAL PROGRESS IN FY 1968

Field Studies

Collection of agricultural samples was phased out with the mid-year, low-temperature feeding trial in January 1968. Approximately half the samples projected for the study, in FY 1962, have been submitted for radiochemical analyses. Three hundred samples were analyzed for Cs^{137} and K^{40} by Battelle-Northwest and the data should be reported before the end of the FY. One man was assigned to a support program initiated in this Section to analyze feeds and milk from each of the 37 feed trials. The analyses are for stable element congeners of the radionuclides reported by the Radiochemical Problems Section for samples from the feeding trials.

Differential moisture stress treatments were applied to 18 water-tight enclosures at NTS during the summer of 1967. Periodic measurements were made of the physiological response by plants undergoing different moisture stress treatments, and of light intensity, wind run at 3 elevations, and water input to the enclosures. Soil moisture and soil temperature sensors were reviewed and repaired on the 68-plot network maintained by Dr. Beatley.

Laboratory Studies

More than two thirds of the Section manpower was expended in manual data reduction this year. The mean transfer coefficient for potassium, in 20 feeding trials, was 0.42 percent of the dietary intake, similar to the transfer coefficient reported for Cs^{137} previously. The maximum secretion of potassium into milk occurred in midsummer as does that of Cs^{137}. Data for two moisture stress experiments was subjected to information theory analysis and manuscripts are in preparation.

15. EXPECTED RESULTS IN FY 1969

Data reduction of radiochemical analyses from the past three years and statistical interpretation of the results will be performed. Material to complete four manuscripts is in process.

16. EXPECTED RESULTS IN FY 1970

Most of the manpower will be applied to preparing data for publication. A cross-over design of irrigations will be applied to plots initially treated at NTS in 1967, to verify observations made then.

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

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

4. Date Prepared:
April - 1968

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 1968	FY 1969	FY 1970
(a) Scientific	6	6	5 1/2
(b) Other Tech.	1/4	1/4	1/4
Total	6 1/4	6 1/4	5 3/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 77,900	$ 82,100	$ 74,700
(b) Materials & Services	8,900	4,700	7,200
(c) Indirect Expenses *	(6%) 59,600	(6%) 60,400	(5%) 54,000
Total	$ 146,400	$ 147,200	$ 135,900

* 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 1968

Life spans of Dipodomys and Perognathus in the Mojave desert. N. R. French, B. G. Maza and A. P. Aschwanden. J. Mammalogy 48: 537-548 (1967).

^{90}Sr in California Mule deer. N. R. French and H. D. Bissell. Health Physics 14. (1968).

A miniature thermoluminescent dosimeter and its application in radio-ecology. A. C. Lucas and N. R. French. In F. H. Attix (editor). Luminescence dosimetry. AEC Symposium Series No. 8. 514 p. 1967.

Comparative dectrocardiography of the Squamata. R. K. Mullen. Physiological Zoology 40: 114-126. 1967.

12. SCOPE OF THE PROJECT

The objectives of research conducted in the Radiation Ecology Section are: (1) to evaluate effects of low level chronic exposure to gamma radiation on mammal populations, and (2) to evaluate effects of environmental factors, biotic and physical, on desert rodent populations.

Life span, fertility, and age structure of irradiated rodent population are being evaluated in the field and in the laboratory. These are the factors that determine rate of increase or decrease of an interbreeding group of animals. Comparisons will be made with control populations.

The techniques of systems ecology are being utilized to investigate relationships of elimatic variables, primary production, and energy requirements, to density of the rodent population.

Results of these investigations will provide insight into the consequences of long continued irradiation at sub-lethal levels, when exposure is to the entire interbreeding population over several generations. It will be determined if environmental factors augment the effects of radiation. The relative importance of measured environmental factors in controlling density of rodent populations should emerge.

13. RELATIONSHIP TO OTHER PROJECTS

In the Rock Valley study area, where our field investigations are conducted, other investigators of this laboratory are studying reptiles, invertebrates, and plants. Demographic analysis of small mammal populations is being conducted at Oak Ridge National Laboratory, Savannah River Operations, and the University of Pittsburgh. Effects of radiation on laboratory colonies of native species of rodents is under investigation at the University of Georgia, Northrup Space Laboratories, Argonne National Laboratory, and Battelle Northwest.

14. TECHNICAL PROGRESS IN FY 1968

Continuous low-level gamma irradiation of a laboratory colony of deer mice, Peromyscus maniculatus, has shown them to be sensitive to an exposure rate of 1 r/day. The intrinsic rate of natural increase, r, was reduced in animals irradiated as adults, and further reduced in the second generation, also irradiated throughout their life spans. The

greater reduction of r in the second generation is a result of additional mortality in the sensitive pre- and postnatal periods of life. Life span was shortened and fertility was reduced by irradiation.

Rodent populations in fenced study areas at the Nevada Test Site had shorter life spans in the irradiated plot, where the average dose rate to animals was 1 r/day. It has not yet been conclusively demonstrated that this is a direct result of radiation exposure.

The utilization of heavy isotopes (oxygen 18 and duterium) to estimate CO_2 production, and hence energy requirements of animals, is progressing in spite of persistent mechanical difficulties with the mass spectrometer. Determinations are not yet sufficiently accurate for the method to be applied to free-living animals.

15. EXPECTED RESULTS IN FY 1969

A computer program has been developed to illustrate the long-term consequences of altered fertility and survival of a rodent population. It is based upon life table data and age specific fertility, and allows for variable length breeding seasons. The program is being modified to permit probabilistic introduction of environmental effects each breeding season using the techniques of systems analysis. The model will be helpful in understanding the relative importance of various factors in determining the success or density of the rodent population.

The radiation source in Rock Valley should be moved to a fenced control plot (Plot C), and the irradiated plot should be made a control. This switch will enable further evaluation of radiation effects on rodents and plants. It will provede the final evidence as to whether or not the differences which have been observed in the plots, such as reduced life span in the irradiated rodent population, is a result of irradiation.

16. EXPECTED RESULTS IN FY 1970

Evaluation of effects of chronic low level exposure to radiation on the desert ecosystem will continue.

Determinations of energy requirements of free-living rodents in the desert will be associated with energy production (primary production) of the desert in an effort to discover factors controlling rodent numbers.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Ecology of the Nevada Test Site

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

4. Date Prepared:
April - 1968

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: 1962 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3 1/2	4	4
(b) Other Tech.	1/4	1/4	1/4
Total	3 3/4	4 1/4	4 1/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 36,600	$ 44,700	$ 47,200
(b) Materials & Services	5,700	3,700	3,200
(c) Indirect Expenses *	(3%) 29,800	(3%) 30,200	(3%) 32,400
Total	$ 72,100	$ 78,600	$ 82,800

* 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 1968

Survival of winter annuals in the northern Mojave Desert. Ecology 48: 745-750. 1967.

Ecology of the Nevada Test Site V. Winter annual survival data. UCLA 12-650. Univ. of California, Los Angeles. 1967.

12. SCOPE OF THE PROJECT

The program, inititated in the spring of 1962, has consisted primarily of field studies at the Nevada Test Site designed to establish certain biological and environmental relationships in the principal ecosystems, an understanding of which is necessary to interpretation of the effects of nuclear and other testing on the native desert species and communities. The period of data collections (6 years) is expected to be over by autumn, 1968.

13. RELATIONSHIP TO OTHER PROJECTS

Ecological studies conducted on other AEC-DBM contracts, and desert biological and environmental studies in a number of institutions, are directly or indirectly related to the plant, animal, and environmental studies conducted on this project at the Nevada Test Site.

14. TECHNICAL PROGRESS IN FY 1968

Vegetation studies. Where needed for conclusion of the plant studies, quantitative data will be collected one more season in the spring of 1968 on some of the network of 68 permanent study sites.

Rodent studies. Censusing of the rodents on all plots was conducted for the fourth consecutive year.

Environmental measurements. Records of precipitation, maximum and minimum air temperatures, soil moisture and temperature, and ionizing radiation were collected bi-weekly for the sixth consecutive year on all of the permanent study sites.

Nevada Test Site Herbarium. Around 1600 plant collections from the Test Site were accessioned, and several thousand duplicates prepared for distribution; many of these collections are being reviewed by specialists in the various groups. In the spring and summer of 1968, a final effort will be made to complete the collections from the Test Site and Bombing and Gunnery Range, for publication of a flora of the region.

15. EXPECTED RESULTS IN FY 1969

Field data collections are expected to be completed by autumn 1968, and thereafter full-time will be devoted to data analyses and manuscript preparation.

16. EXPECTED RESULTS IN FY 1970

Data analyses and manuscript preparation will continue, and several publications, including an annotated flora of the Test Site and certain facets of the ecosystem studies, will be completed.

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:
Radioecology

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

4. Date Prepared:
April - 1968

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: 1961 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	4	4	4
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total	$4\frac{1}{4}$	$4\frac{1}{4}$	$4\frac{1}{4}$

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 58,100	$ 57,900	$ 61,500
(b) Materials & Services	4,000	3,900	4,800
(c) Indirect Expenses *	(4%) 39,700	(4%) 40,300	(4%) 43,200
Total	$101,800	$102,100	$ 109,500

* 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 1968

Acute gamma irradiation experiments with the lizard Uta stansburiana. Radiation Res. 31: 27-35. (With J. R. Lannom, Jr., M. J. Kania and B. W. Kowalewsky).

A model of secondary production by the lizard Uta stansburiana. Bull. Ecol. Soc. America 48 (2): 60. (With B. W. Kowalewsky and E. A. Carl).

A comparison of some demographic attributes of irradiated and non-irradiated populations of Uta stansburiana in Nevada. Bull. Ecol. Soc. America 48 (3): 137-138. (With G. A. Hoddenbach and J. R. Lannom, Jr.).

Preface to the American Society of Zoologists 1967 Refresher Course, "Energy flow and ecological systems." American Zoologist, Feb. 1968.

Radiation doses sustained by lizards in a continuously irradiated natural enclosure. Ecology (in press). (With J. R. Lannom, Jr.).

12. SCOPE OF THE PROJECT

The effects of low levels of external irradiation on natural populations of animals are not known. The Rock Valley study entails the continuous irradiation of natural populations in such a way that all individuals receive approximately equal doses. One objective of this study is to define the effects, if any, of continuous exposure to low levels of gamma radiation on populations of lizards. The Rock Valley study also considers the impact of chronic irradiation on the whole community as an entity, both in terms of its organization and function. Special emphasis is being placed on the comparative energy metabolism of irradiated and non-irradiated communities.

In the Rock Valley study, the primary objective is to seek evidence of modification of population parameters by irradiation, and to correlate any observed changes with dose experience. Hence, it is important to evaluate the dose experience of individuals as precisely as possible. Although free-air doses will be documented in both study areas, these data will not be applicable to the dosages experienced by animals. Therefore, tissue doses to individual organisms will be estimated by the use of small thermoluminescent dosimeters.

In keeping with the community-oriented focus of the Rock Valley program, studies of annual changes in the reproductive performances of lizard populations are being made. The causes of year to year differences in egg production and survival are being investigated in order to clarify the mechanisms regulating population size, and to yield data necessary to understand the energy dynamics of the community.

13. RELATIONSHIP TO OTHER PROJECTS

The Rock Valley project uses facilities designed by Dr. Norman R. French. Whereas Dr. French is investigating the long-term effects of irradiation on small mammals, the study discussed here pertains to lizards. Dr. Donald Tinkle at the University of Michigan has carried out experiments on the effects of X-irradiation on lizards (Uta stansburiana). Studies on the effects of X-irradiation of toads are being carried out by Dr.

Frank Blair at the University of Texas. Studies of the effects of chronic gamma irradiation on selected arthropods have been conducted at Brookhaven National Laboratory under the direction of Dr. George Woodwell. Dr. Frank Golley and Dr. Ernest E. Provost at the University of Georgia's Savannah River Project are investigating radiation effects on various species of wild mammals.

Additionally, work related to one or another portions of the above is being carried out 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
Colorado State University, Fort Collins, Colorado
University of Washington, Seattle, Washington
Brigham Young University, Provo, Utah
University of Nevada, Reno, Nevada
Puerto Rico Nuclear Institute, San Juan, Puerto Rico
Battelle-Northwest, Richland, Washington
National Reactor Testing Station, Arco, Idaho

14. TECHNICAL PROGRESS IN FY 1968

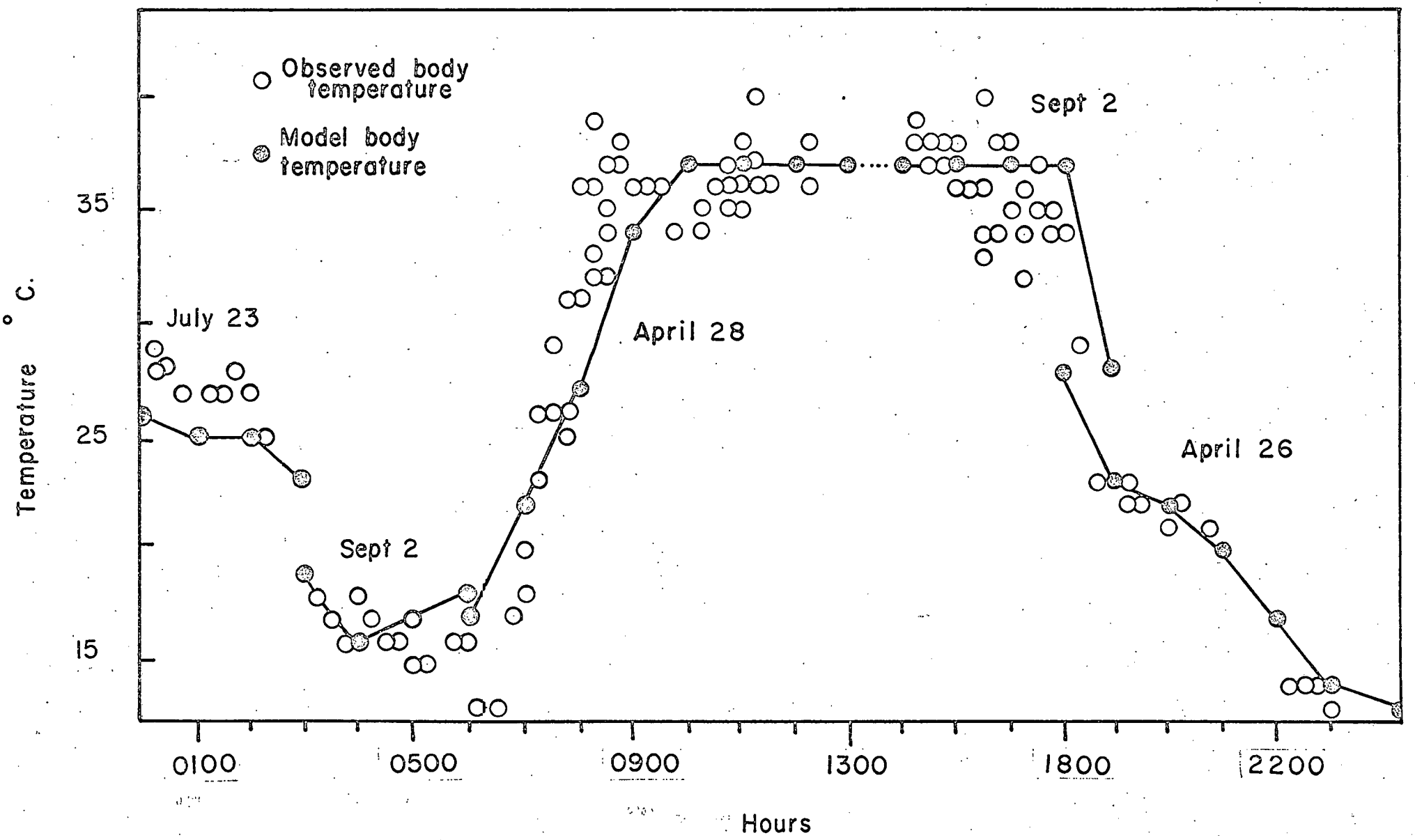
1. Work has continued on a simulation of energy utilization and production by Uta stansburiana. Our model takes into account (i) the annual cycle of density and biomass, (ii) the physiology of respiration, as a function of activity and temperature experience, and (iii) the caloric content of Uta. The density of Uta varies between plots and from year to year. Estimated densities as low as 14 and as high as 29/acre were recorded in 1965 and 1966. For the purpose of the energy flow model, a spring density of 20/acre was assumed. Summer recruitment was estimated from known schedules of age-specific fertility. During August and September population density may approach 50/acre. The demographic basis of our model is a series of month-by month estimates of population size, coupled with the weight distribution of the population, and taking into account monthly mortality rates.

The hour by hour and day by day temperature experiences of individual Uta have been analyzed from a series of about 1600 measured body temperatures, These temperatures were recorded at times for which corresponding air temperatures and sun conditions were known. Given certain other assumptions, these data were combined in an empirical model capable of predicting body temperatures as a function of air temperature and pyrheliometer data.

During the warm times of the year the model predicted body temperature regimes which accorded closely with field measurements (Figure 1). The performance of the model was less impressive during the winter months, but still adequate for our purposes.

The metabolic rate of Uta, as a function of body temperature and activity, was estimated from the regression functions of Roberts Proctor.

Calorimetry has been carried out on tissues of Uta stansburiana. We found that lyophilized tissue was about 0.3 the weight of live tissue,


Observed body temperature
Model body temperature
35
25
15
° C.
Temperature
July 23
Sept 2
April 28
Sept 2
April 26
0100
0500
0900
1300
1800
2200
Hours

and that the caloric content of lyophilized tissue was about 5275 ash-free cal/g. Live weight of Uta is then about 1570 cal/g.

A tentative estimate of energy flow in Uta during 1965-66 of 664 Kcal/acre/year was derived. Unfortunately it is presently impossible to evaluate this estimate in terms of overall community function. A study of the kangaroo rat (Dipodomys) in Arizona indicated an annual flow of about 25,000 Kcal/acre, or about 40 times that of Uta in Nevada. However, it must be borne in mind that about 98% of the energy utilized by mammals is by respiration, with a correspondingly small contribution to net secondary production. Our estimate of the budget of Uta indicated that only about 80% of the total energy flow was energy of respiration. Hence, about 20% of the total energy utilized was expressed in the production of new tissue available for other consumers. Viewed in this way the role of Uta, and lizards in general, may not be negligible.

2. Individuals of Uta stansburiana in Rock Valley receive about 400-2000 rads/year, depending on their location within the experimental area. The maximum exposure rate is during the summer, when essentially all incident gamma radiation is absorbed. We judge that most individuals receive, on average, about 2-4 rads/day.

These exposures are low compared with chronic dose regimes which have had clear-cut effects on the fertility and life span of animals. Studies of chronically irradiated laboratory populations of mammals have generally failed to show effects on life span when the weekly doses were less than 10 rads, although recent laboratory work by Dr. French indicates an impairment of fertility and a life-shortening effect in white-footed mice (Peromyscus maniculatus) exposed to life-time chronic doses of around 1 rad/day.

Our studies of Uta indicate that, after three years of almost continuous radiation exposure, the experimental population continues to perform in a manner which cannot be distinguished from that of populations in the three control areas. All populations increased appreciably (roughly 40%) between 1966 and 1967. The increase of the irradiated population was not judged to be significantly different from that observed in the other three areas. The sex ratio of the irradiated population is apparently identical with that of the other three populations. Comparisons of the age distributions of irradiated and control populations show that the maximal life span of the irradiated animals (about 4 years) does not differ from that of the controls. Tests of the relative proportions of individuals in each age class do not show significant differences between populations. Our comparisons of population age structures are not enormously sensitive, but it is at least true that if there is a life-shortening effect on Uta it is being expressed at a fairly subtle level.

Year	Age (months)	Controls	Experimental	χ^2 (2x3 contingency table, 2 d.f.)	P
1966	8	196 (201.0)	143 (138.0)		
	20	64 (59.9)	37 (41.1)	1.147	0.5 - 0.7
	32+	14 (13.1)	8 (8.9)		

1967	8	239 (233.8)	118 (123.2)		
	20	34 (39.3)	26 (20.7)	2.409	0.3
	32+	10 (9.9)	5 (5.1)		

Comparisons of age distributions of Uta stansburiana observed in three control areas and one irradiated area in Rock Valley, Nevada, and 1966 and 1967. Oldest age group includes a few individuals 44 months of age. Expected values are given in parentheses.

3. A third area of endeavor during 1967-8 was the development of better data storage, editing and retrieval procedures. Over 13,000 records of Uta alone have now been accumulated and mechanical data reduction procedures are imperative. Some of the problems faced included: conversion of campus computing facility from an IBM 7094 to an IBM 360/75 system, rewriting some existing Fortran IV programs in another language, development of a blocked tape format and the preparation of a series of 3 tapes to permit periodic updating of records, the preparation of a special editing program capable of screening and adding new records to the data matrix. Some of these steps have been time-consuming but it is believed that these procedures will be ultimately justified.

15. EXPECTED RESULTS IN FY 1969

Sampling of lizards in four 20-acre areas in Rock Valley will be continued. Four years of observations have revealed no obvious direct effects due to radiation exposure, and this is in keeping with original predictions. The lack of any strong influences of the irradiation on plants suggests that no indirect effects can be expected in the near future. It is true that the available data have not been analyzed to the fullest degree possible, and work along this line will be continued. Work on the mean clutch size of Uta will be continued in order to trace year to year changes in the rate of increase.

Work on a general hypothesis explaining density changes in Uta will be continued. In a general way, regulation is visualized as an interaction between available food and the density-dependent factors influencing survival of young females between the time of birth and the first breeding. It has already been shown that from 70-80% of the reproduction achieved by the population is accomplished by individuals 9-11 months of age. Spring densities, then reflect primarily (i) the fecundity of first year females in the preceding year (itself an expression of clutch size and number) and (ii) survival of young between July and the following spring. Clutch size and number is probably tied to available food, although changes in the average size of breeding females may play a lesser role. To actually demonstrate changes in fecundity under conditions of differing food availability would be difficult, but might be achieved by experimental alterations of the environment. Presumably the fundamental variable is production by annuals (and shrubs?), with prey density in some way linked to primary production. Whether there is a lag effect, i.e., whether the food conditions during one season affect events during the next is not known.

Presently, the critical deficiency in the Rock Valley program is the absence of regular quantitative sampling of annuals and shrubs, and the lack of clearly demonstrated annual changes in the biomass of arthropods serving as food for insectivorous lizards. Attempts will be made to

stimulate the acquisition of data pertaining to these problems.

Work on the model of Uta energetics will be continued. Two avenues of advance are envisioned: (i) manipulation of the model inputs in order to determine the total range of plausible variation in outputs, given prescribed uncertainites in the input parameters, and (ii) generalization and simplification of the model insofar as is possible. Bomb calorimetry will be expanded to include other important Rock Valley species. Further acute irradiation experiments with lizards may be conducted if opportunity arises.

16. EXPECTED RESULTS IN FY 1970

The program in FY 1969 will be exclusively concentrated in Rock Valley, with primary emphasis on Uta stansburiana and the factors influencing its dynamics. Procedures will be essentially as in the past.

It is possible that the radiation source will be moved to another one of the four areas under investigation. This procedure may alleviate some of the difficulties in interpreting subtle differences between the areas which cannot be definitely ascribed to the irradiation.

The possibility of experimentally altering small study areas in order to test certain environmental relationships will be explored.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE — Field Office

BIOLOGY AND MEDICINE — Program

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

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

2. Project Title:
Physiology of Mineral Accumulation in Plants

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Owen R. Lunt

8. Project Term:
From: 1966 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	1 1/2	1	2
(b) Other Tech.	1/4	1/4	1/4
Total	1 3/4	1 1/4	2 1/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 17,000	$ 15,000	$ 24,100
(b) Materials & Services	3,100	2,600	5,200
(c) Indirect Expenses *	(1%) 9,900	(1%) 10,100	(1%) 10,900
Total	$ 30,000	$ 27,700	$ 40,200

* 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 1968

Wallace, A., R.T. Ashcroft and O.R. Lunt: Day-Night Periodicity of Exudation in Detopped Tobacco, Plant Physiology 42, No. 2, 238-242 (1967).

Youngner, V.B., O.R. Lunt and F. Nudge: Salinity Tolerance of Seven Varieties of Creeping Bentgrass, Agrostis palustris Huds, Agron. J. 59, 335-336 (1967).

Kaempfe, G.C. and O.R. Lunt: Availability of Various Fractions of Urea Formaldehyde, Jour. Agric. & Food Chem., 15(6), 967-971, (Nov.-Dec. 1967).

Jones, R. C. Wyn and O.R. Lunt: The Function of Calcium in Plants, The Botanical Review, 33, No. 4, (Oct.-Dec., 1967).

Lunt, O.R. and J.G. Seeley: Irrigation of Turfgrass, Flowers and other Ornamentals, in Irrigation Monography, Chapter 39 in Amer. Soc. of Agron., 753-768, (1967).

12. SCOPE OF THE PROJECT

Soil-water-plant relations. The water relations in arid region ecosystems are dominant factors and an understanding of the adaptations and physiological response of plants under the prevailing conditions is basic to evaluating the additional effects which radiation or radionuclides may introduce into the system. It is proposed to focus attention primarily at this time on the perennial shrubs. Attempts will be made to measure diffusion pressure deficits of the plant fluids by field techniques at various seasons of the year, relating these measurements as much as possible to soil moisture conditions. Likewise, attempts will be made to evaluate other physiological functions such as photosynthesis, respiration and transpiration to DPD. At appropriate stages in the study the sensitivity to radiation as a function of physiological condition of the plant will be investigated.

In the area of mineral nutrition two areas of research particularly invite investigation as being contributory to an understanding of parameters functioning in the ecosystem. One is the comparative mineral requirements and tolerances of plants which have evolved under arid conditions. There is a great temptation to translate nutritional physiology from the more familiar agricultural plants to those which have evolved under arid conditions. Since environmental conditions are strikingly different, it would not be surprising to find differences which would provide means for studying physiological diversity. Secondly, within this broad context, it is proposed to direct attention first to calcium requirements and relations. This would appear to be a fruitful avenue of investigation since calcium minerals are typically very high in arid region soils, often forming thick mineral deposits of $CaCO_3$ or $CaSO_4 \cdot 2H_2O$ on the underside of surface rocks, in channels or around roots. In crop plants, minimum calcium requirements vary from about 200 ppm to about 1%. Calcium also interacts with micronutrient functions and active transport process. Basic information on mineral nutrition contributes to an understanding of the accumulation of radionuclides.

Work will be undertaken to apply a very new and extremely attractive field technique for detecting nitrogen fixation in N.T.S. soils, microorganisms,

and plant species. It has recently been recognized that nitrogenase, the enzyme responsible for N_2 fixation, will reduce many triple bonded molecules other than N_2. The method makes use of the reduction of acetylene to ethylene by the enzyme. Ethylene can then be detected with great sensitivity by gas chromatography. Obviously the assessment of routes of introduction of fixed nitrogen into the ecosystem is of considerable interest.

13. RELATIONSHIP TO OTHER PROJECTS

Research activities will be coordinated with and involve the cooperation of investigators in the Environmental Radiation Division whose interests are in the areas of soil-water-plant relations and mineral accumulation, transport and function.

Related studies are conducted at the following locations:

Duke University, Durham, North Carolina. AT(40-1) 1827, 3094.
Ecology Section, ORNL, Oak Ridge, Tennessee, W-7405-ENG-26.
Biology Department, Battelle-Northwest Laboratories, Richland, Washington. AT(45-1)1350.
U.S. Department of Agriculture, Beltsville, Maryland. AT(49-7)-1.
Washington State University, Pullman, Washington. AT(45-1)1543.
University of Arizona, Tucson, Arizona. AT(11-1)947.

14. TECHNICAL PROGRESS IN FY 1968

Soil moisture response studies have not reached the point where we feel we have publishable data. The species under investigation is Larrea which seemingly displays considerable genetic variability. Thus, it is important to work with carefully matched clones. Qualitatively, the species responds rapidly to moisture after existing in a more or less dormant condition due to severe moisture stress. Studies are also in progress on aeration requirements of Larrea. No data is available on this characteristic of arid region species and it may be a significant ecological parameter affecting rooting depth.

Work on calcium function in plants (largely supported by an NSF Grant) is partially summarized in the publication by Wyn-Jones and Lunt. Other articles are in preparation. Work since last year's report centered on an attempt to quantitatively localize calcium in the cell with the electron microprobe. This work was interrupted by the necessity of Dr. Wyn-Jones to return to England unexpectedly. The completed work will be published in a series of papers. The publications other than the one by Wyn-Jones and Lunt are the result of previous work.

15. EXPECTED RESULTS IN FY 1969

It is expected that our current studies on water response and aeration studies will have reached their initial objectives of quantitatively defining at least the gross aspects of the response of Larrea to soil moisture and root requirements for oxygen.

It is also anticipated that considerable progress will have been made on exploring the sources of nitrogen input into the N.T.S. ecosystem.

16. EXPECTED RESULTS IN FY 1970

The direction of work in FY 70 will be influenced by results obtained in FY 69. It is anticipated that major efforts will continue to be in the general area indicated for next year.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title:
Radioecology-Arthropods

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UC-Riverside and Nevada Test Site

7. Person in Charge:
Vernon M. Stern

8. Project Term:
From: 1966 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2	1 1/2	1 1/2
(b) Other Tech.	1/4	1/4	1/4
Total	2 1/4	1 3/4	1 3/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 24,300	$ 20,500	$ 21,900
(b) Materials & Services	2,100	2,800	2,900
(c) Indirect Expenses *	(2%) 19,900	(1%) 10,100	(1%) 10,800
Total	$ 46,300	$ 33,400	$ 35,600

* 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 1968

Interrelations of the aphid, Ocyrthosiphon pisum and its parasite Aphidius smithi in a stable environment. R. van den Bosch, C. Lagace, and V. M. Stern. Ecology 48(6): 993-1000. 1966.

Ecological approach in integrated pest control. V. M. Stern and J. M. Franz. Proc. FAO Symposium on integrated pest control. Rome, Italy. 2: 7-10. 1966.

Discussion on ecological approach in integrated pest control. V. M. Stern and J. M. Franz. Proc. FAO Symposium on integrated pest control. Rome, Italy. 2: 33-5. 1966.

The essential role of economic-injury population levels, and recommendations to the Director-General of FAO. V. M. Stern and (Panel Members). Report of the FAO Symposium on integrated Pest Control, pp. 22-23; 39-40. Rome, Italy. 1966.

Lygus control by strip cutting alfalfa. V. M. Stern, R. van den Bosch, T. F. Leigh, W. Sallee, O. MacKutchen and M. Garber. Univ. of Calif. Ext. Serv. Circular No. AXT-241, 13 pp. 1967.

Control of aphids on barley. V. M. Stern and W. R. Bowen. Calif. Agric. 21(3): 14-15. 1967.

Cultural Control of Lygus. Cotton Insect Control. V. M. Stern, A. Mueller, T. F. Leigh and R. van den Bosch. Mimeo. Division of Agricultural Sciences, Univ. of Calif., pp. 23-8. 1966.

12. SCOPE OF THE PROJECT

The original project at the Nevada Test Site was designed to study the effects of chronic low level radiation on insects. This study has been in progress for nearly 3 years and under the direction of the present project leader for a year and a half.

There is ample evidence in the literature to show that insects can generally withstand a very high degree of radiation without abnormal physiological effects. However, in most studies there has been little correlation between ecologically related insect species. In addition, most of the past work and research currently underway mainly deal with a few pest species. More basic knowledge is needed to correlate ranges of tolerance between arthropods in the same ecosystem. Not only is the effect of chronic low level gamma radiation on arthropods needed but also the effects of high short term beta and gamma radiation as suggested by Teresi and Newcombe (NRDL - ONR - OCD Work Unit No. 3145A).

Because of the recent test in the Plowshare Program there is now a unique opportunity to expand the present study to include the effects of higher dosages of short term radiation on related arthropod populations in the U20 area at NTS. In the expansion of the present study of short term high dosage radiation certain emphasis will be shifted to laboratory experiments to simulate results of predicted fallout on the ecosystem.

In regard to the Rock Valley study the present insect population density changes in the chronic low-level radiated area are not expected to differ significantly from those in the control areas. In fact, changes in population density are probably more likely to be those of varying climatic changes or from indirect effects owing to long-term radiation induced changes in the vegetation or on populations of mammal or reptile predators rather than from direct radiation effects on the arthropods.

13. RELATIONSHIP TO OTHER PROJECTS

Field investigations at NTS are correlated with other investigators from the Environmental Radiation Division, UCLA. These include studies on mammals, reptiles and plants. Further studies on arthropods are being carried on by Dr. D. A. Crossley at the Oak Ridge National Laboratory as well as investigations of the effects of radiation on insects with different chromosome volume by Dr. H. Cromroy at the University of Florida (NRDL - OCD). In addition a recent project has been initiated by NRDL - ONR on the hypothetical effects of radiation on arthropods under conditions of various MT surface bursts. The USDA is also studying and has already developed techniques of applying practical and peaceful uses of atomic energy to control insect pests by male sterilization.

14. TECHNICAL PROGRESS IN FY 1968

Arthropod sampling was continued in the four plots at Rock Valley. Techniques and procedures of marking various beetles were conducted with each sampling period. This procedure proved highly valuable and re-capture of beetles showed that a number of Eleodes armata adults that were tagged in the early spring 1965 survived the winter of 1965, 1966 and 1967. There were also other species that survived the winter and were recaptured during the summer 1967.

Research was continued in 1967 by doubling the sampling frequency in each of the four Rock Valley plots. Small changes in seasonal activity and behavior were thus more noticeable. Activity peaks apparently vary from one year to another and appear to be closely tied to the physical conditions of this area.

Continuous recordings of soil temperature at the surface and at 6 and 9 inches below ground level were accumulated as well as wind speed at 1 ft. These data are especially critical during the hot summer months when soil surface temperatures rise above the highest tolerance limits for the arthropods under study. Temperatures below the surface are within the tolerance limits of most arthropods. Other weather data was taken by the AEC and this will help strengthen the data on the arthropod project.

Studies were also conducted on the insect feeding habits of lizards. In this regard gut contents are being analyzed at various times of the year and the insect species found are being identified to the family level.

15. EXPECTED RESULTS IN FY 1969

The 1968 program at Rock Valley will be reduced to sampling arthropods in each of the 4 test plots once per month. There has been no detectable change in the arthropod populations due to low level radiation at Rock Valley and there will be an expansion of research efforts into the U20

area in conjunction with the Plowshare Program. These efforts will be related to arthropod activity in disturbed situations. Mortality effects and recolonization of the disturbed areas will be followed as well as the immediate effects of high level radiation from fallout.

It is anticipated that some provision will be made for a greenhouse at Mercury. These studies will relate to the biology of a number of insect species to better interpret results in the Rock Valley study as well as study the effects of high-dosage radiation on the biology of ecologically related species in the U20 area.

16. EXPECTED RESULTS IN FY 1970

It is anticipated that all phases studied in 1969 will be actively investigated in 1970.

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 Physiological Ecology

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Arthur Wallace

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3	4	4
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total	$3\frac{1}{4}$	$4\frac{1}{4}$	$4\frac{1}{4}$

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 48,500	$ 62,300	$ 63,700
(b) Materials & Services	4,500	9,700	5,800
(c) Indirect Expenses *	(3%) 29,800	(4%) 40,300	(4%) 43,200
Total	$ 82,800	$ 112,300	$ 112,700

* 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 1968

Germination of Russian Thistle Seeds. W. A. Rhoads, E. F. Frolich, and A. Wallace. Calif. Agriculture 21(7): 2.

Behavior of rare earth elements in plants and soils. E. M. Romney, A. Wallace, and R. A. Wood. Amer. Assoc. Advan. Sci. June 19-22, 1967. (Abstract) (1967).

Chelating agent effect on availability of easily reducible Mn^{54} and manganese in different soils by corn and bush beans. A. Wallace. Amer. Soc. Agron. Ann. Meet., Nov. 1967, College Park Md. (Abstract) (1967).

Effect of phosphorus and other deficiencies on leaf temperature in tobacco. A. Wallace, E. F. Frolich, and R. T. Ashcroft. Agron. Jour. 59: 386. (1967).

Effect of bicarbonates on exudation in detopped tobacco. A. Wallace, M. W. M. Leo, and R. T. Ashcroft. Proc. Ann. Meet. Amer. Soc. Plant Physiol. Aug. 28-31, 1967, Vol. 42, Suppl. Texas A&M, College Station, Texas. (Abstract) (1967).

Retranslocation of Rb^{86}, Cs^{137}, and K to new leaf growth in bush beans. A. Wallace. Plant and Soil (in press) (1968).

Germination behavior of Salsola as influenced by temperature, moisture, depth of planting, and gamma irradiation. A. Wallace, W. A. Rhoads, E. F. Frolich. Agron. Jour (in press) (1968).

12. SCOPE OF THE PROJECT

The purposes of this research project are to obtain fundamental plant physiological and biochemical information concerning species native to the Nevada Test Site, to evaluate their susceptibility and response to ionizing radiation, and to study radionuclide and mineral element cycling in the species.

Specific research includes field studies of effects of ionizing radiation, field and laboratory studies of species susceptibility to radiation, effects of altered environment (mineral nitrogen, chelate, water, removal of dominant species, pesticides, radionuclide contamination) on the physiology and ecology of chosen sites, glasshouse and laboratory studies of plant species to elucidate peculiarities of their survival adaptations to the desert environment, and studies of fundamental mineral nutritional characteristics of the species native to the desert.

Long term irradiation studies of the native shrub species in Rock Valley continue, and the data accumulated between 1964 and 1966 inclusive are currently being evaluated. This study is an essential part of the Rock Valley program and is a parallel and complement to the mammal and reptile studies there.

New attention in the Rock Valley area will be directed toward an assessment of annual plant productivity and mineral composition. From biomass data and mineral analyses, the contribution of these plants to mineral cycling will be calculated. Mineral cycling studies will be extended to

as many other components of the ecosystem as feasible and in cooperation with as many other sections as practical. It is intended to use various radionuclides in the study of mineral cycling including Cs^{137}, Ca^{45}, P^{32}, Zn^{65}, C^{14} plus the stable N^{15}.

13. RELATIONSHIP TO OTHER PROJECTS

Research is corrleated with other sections of the Environmental Radiation Division: Soil Factors Section, H. Nishita (480310); Plant Factors Section, E. M. Romney (480311); Environmental Decay Section, H. A. Hawthorne (480320); it is also part of the Rock Valley project being carried on by Radiation Ecology Section, N. R. French (480341); Radio-ecology Section, F. B. Turner (480343); Desert Ecology, J. C. Beatley (480342).

Related studies are conducted at other laboratories: U. S. Department of Agriculture, Beltsville, Maryland AT (4907) 1; Utah State University, Logan, Utah AT (11-1) 1287; Plant Physiology Section, Battelle-Northwest Laboratories, Richland, Washington; University of California at Los Angeles AT (11-1) 134 Project 51; Michigan State University, E. Lansing, Mich. AT (11-1) 888; Dept. of Hort. Science, University of California, Davis, Calif. AT (11-1) 134, 38; Oregon State University, Corvallis, Oregon, AT (45-1) 1547; Tuskegee Inst. Alabama AT (40-1) 2749; University of California, Agricultural Experiment Station, Riverside, Calif. These projects are also closely related to those being carried out by Sparrow's group at Brookhaven National Laboratory, by Robert Platt and his co-workers, Emory University, and by Auerbach and his colleagues at Oak Ridge National Laboratory.

14. TECHNICAL PROGRESS IN FY 1968

Shrub data from the plots in Rock Valley for 1964 through August of 1967 have been organized into a report that is being prepared for publication. The study covered those aspects of shrubs which are most readily available for evaluation, i.e. growth of branches, twigs and leaves; and the production of flowers and fruit. This was accomplished by taking, during the growing season, a large number of measurements of lengths of branches, counts of leaves, flowers, fruits; and in some species, measurements of internodal lengths, and the development of nodes are also made. Very large numbers of measurements were required because of the variability of these characteristics due to environment and fundamental constitution of those shrubs being investigated.

As a complement to the above study, other studies are underway among shrubs of a different ecosystem, within the Test Site, at an elevation of 6000 feet, which is 2000 feet above the Rock Valley elevation. This study is of broader scope in that it also considers other radiation than gamma radiation. In the northwest corner of the Test Site there are localized areas in which radioactive materials have been scattered in the open environment. This provides a unique opportunity for study of the cumulative effects of mixed sources of irradiation on and among the shrubs. Phenology, seed germination, and physiological studies will be made of plants in these areas. In support of this study a portable Co^{60} source has also been and will be further used in order to determine the relative sensitivities of those shrubs under consideration to a central

source of gamma irradiation.

Seeds and seedlings (or rooted cuttings) of several species native to the Nevada Test Site were irradiated and responses were studied. There appear to be great differences among sensitivity. These studies complement those being made in the field both with the portable 100 curie Co^{60} source and with sites which have been damaged in the nuclear testing programs.

A system of test plots was established (in cooperation with Dr. Romney) near Mercury at the Nevada Test Site to study factors which limit primary productivity in the ecosystem involved and also to determine some of the species interrelationships. Environmental manipulations are being used on the plots and include irrigation, nitrogen and phosphorus fertilization, metal chelate additions, and insect control. These plots are being censused for shrub populations.

Germination and root cutting characteristics of many plant species have been determined and these are providing plants for laboratory and glasshouse studies.

Some phenological characteristics of shrub species were determined with the goal of being able to study mineral element cycling. Age of plants, leaf fall, and fruit production were studied. Characteristics of many species have been documented. A semi-detailed study of nitrogen turnover in the ecosystem near Mercury was made. Some initial plant mineral analyses were made. These studies indicate that phosphorus and not nitrogen may be a limiting factor in primary productivity.

Some physiological studies of irradiation effects are in progress. Salt uptake, incorporation of P^{32} into RNA, chlorophyll synthesis, and water movement in detopped plants at various levels of irradiation with the laboratory Co^{60} source are under investigation. Those effects which are of ecological interest are of most concern.

15. EXPECTED RESULTS IN FY 1969

The completion of the data analyses of the shrubs in Rock Valley is expected. This will include an assessment of the data for the irradiated vs. the non-irradiated plot.

More information about the sensitivity to ionizing radiation of plant species from the Nevada Test Site and their physiological responses to ionizing radiation is expected. Laboratory studies will complement field studies. Both germination and physiological studies will be made of plant materials from the Cabriolet Test.

A detailed statistical shrub interrelationship study will be made of the plots which were censused in FY 1968.

Studies of plant phenology and plant behavior for several species will be continued in the laboratory as well as in the field.

We expect to initiate a soil survey of major areas at the Nevada Test Site and to do detailed chemical analyses on the soil samples collected.

The data are to be integrated into an ecosystem analysis for each area.

The mineral cycling studies will be intensified. Thousands of samples will be processed and some attempt at synthesis into meaningful cycles will be attempted. Attempts will be made to use these data to help unify the other studies at Rock Valley into an ecosystem approach.

Preliminary data will be available of the studies of limiting factors and responses to environmental manipulation.

16. EXPECTED RESULTS IN FY 1970

It is anticipated that all phases of research done in 1969 will be continued in 1970. The field plot work will be expanded and mathematical treatment of the results will be undertaken. It is anticipated that the development of a glasshouse at the Nevada Test Site will make possible additional output of information on physiology of desert species and on radionuclide cycling. Mineral cycling including carbon, nitrogen and phosphorus cycles under the desert system will be studied.

Further evaluation of environmental influences, that is micro-environmental differences, versus the effects of irradiation are 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 Problems

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Robert A. Wood

8. Project Term:
From: 1956 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	6 1/2	6 1/2	6 1/2
(b) Other Tech.	1 1/4	1 1/4	1 1/4
Total	7 3/4	7 3/4	7 3/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 79,500	$ 81,000	$ 85,700
(b) Materials & Services	17,800	14,000	12,100
(c) Indirect Expenses *	(6%) 59,700	(6%) 60,400	(6%) 64,800
Total	$ 157,000	$ 155,400	$ 162,600

* 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 1968

Solubilization Method for Centi-Gram to Kilo-Gram Plant Samples. U.C.L.A. 12-613, 12 pp., Jan. 1967.

The Sequential Separation and Quantitative Determination of Sr^{90}, Ce^{144}, Cs^{137} and the Stable Elements of Sr and Ca in Plants, Feces, Milk and Water Samples. U.C.L.A. 12-676, 67 pp., Feb. 1968.

Other publications will be found in the programs of the various sections supported by this section.

12. PURPOSE, NEED AND SCOPE

The Chemical Problems Section's primary function is the support of the program's objectives of the Environmental Radiation Division on its research requirements for analytical, inorganic, and radiochemistry. The secondary objective is to conduct research in the development of new analytical techniques and methods relevant to division programs and research requirements.

The third objective is the development of new and unique analytical or counting systems that will enable more rapid and precise analytical analysis. In addition to these principal objectives research is being conducted to develop methods using neutron or charged particle activation techniques for the production of short half-lived radionuclides useful as tracers in medical or ecological research programs (i.e. Mg^{28}, Sr^{87m}, etc.)

Studies are also being conducted to investigate the physiochemical change in soil when subjected to high dose rates of gamma and beta irradiation. The water solubility of the alkali metals, alkaline earth metals and the rare earths (atomic No. 58-71) are presently being investigated with reference to this program.

Other services rendered by this section include the preparation and calibration of counting standards.

Several continuing programs are being carried out jointly with investigators in this Division and other Divisions. The Environmental Decay Section has required large numbers of analysis for Sr^{90}, $CePr^{144}$, Cs^{137} and the stable elements of Sr and Ca. This Section has given support to the Soil Factor Section both in the production and analysis of various radionuclides (rare earth cycling in plants) in ecological cycling studies.

In the past our primary effort has been the determination of Sr^{90}, $CePr^{144}$ and Cs^{137}. We are presently equipped and staffed to perform analysis for all the fission and neutron induced radionuclides.

13. RELATIONSHIP TO OTHER PROJECTS

Analytical work and research of similar nature is being carried on in the following organizations:

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

U.S. Naval Radiological Defense Laboratories, San Francisco, Calif.

Lawrence Radiation Laboratories, Livermore, Calif.

Battelle Northwest Laboratory, Richland, Washington.

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

14. TECHNICAL PROGRESS IN FY 1968

Support Projects:

During the first eight months of fiscal year 1968 approximately 2500 determinations were completed for the analysis of Sr^{90}, $CePr^{144}$, Cs^{137} in plant, milk, feces, bone, GI tracts, soil, muscle and water samples. A more detailed breakdown of samples processed is as follows:

1. Environmental Decay Studies, Dairy Farm Project: Milk, soil, feces and plants were analyzed for Sr^{90}, $CePr^{144}$, Cs^{137}, Zr^{95}, Ra^{226} and the stable elements of Sr and Ca. It is expected that 40% of the determinations run during the next four months of this Fiscal Year will originate from this section.

2. Soil Factors Studies, Plowshare Program: Soil, plant, GI tracts, bone, muscle and vital organs were analyzed for Sr^{89}, Sr^{90}, Ba^{140}, $CePr^{144}$, Cd^{106}, Cs^{137}, Mn^{54} and the stable elements of Sr, Ca, K and Mn. It is expected that 30% of the determinations run during the next four months will originate from this section.

3. Plant Physiological Ecology and Soil Factors Project Plowshare - the investigation of mineral cycling of neutron induced rare earth radionuclides in plants; Tracer solutions of the rare earths are prepared by neutron activation of the stable salts of these elements. Less than 5% of the Laboratory effort will be devoted to this study during the next four months.

4. Chemical Problems, methodology development, instrumentation development and Radiolysis research in complex systems: It is expected that about 25% of the Laboratory effort will be devoted to these investigations during the next four months.

15. EXPECTED RESULTS FOR FY 1969

The determination of the radionuclide content of plant, feces, bone, milk, soil and water samples will be continued at the same level for the next

fiscal year. It is expected that in addition to the continuing cycling studies of Sr^{90} and Cs^{137} in various ecosystems being investigated by this Division, program objectives will be expanded to include the biologically significant neutron induced radionuclides associated with under-ground nuclear events. Several new methods are under development to enable the rapid radiochemical analysis of these nuclides in complex matrices (i.e. plant, soil, milk, etc.). The direct, non-destructive measurement of gamma emitting radionuclides in the various kinds of samples processed by this laboratory will be greatly increased during the next fiscal year. It is hoped that during FY 1969, the need for wet radiochemical analysis of these nuclides will be eliminated, thus, saving the Division considerable funds that are absorbed in wet radiochemical analysis. It is expected that neutron activation, for both trace element analysis and isotopic production will be greatly increased during FY 1969.

16. EXPECTED RESULTS IN FY 1970

No major changes in direction are anticipated although more emphasis will be given to neutron induced radionuclides and mineral cycling thus, requiring the development of new methods and analytical techniques to meet these expanding division research needs. It is expected that the recently installed low background, anti-coincidence gamma detector system will enable the chemistry laboratory to limit its support activities to the analysis of stable elements and pure beta emitting radionuclides. This will permit more effort to be given to the chemically oriented problems that exist in the various research programs within the division.

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:
Quantitative Plant Ecology

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
(To be Named)

8. Project Term:
From: 1968 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	0	3/4	2
(b) Other Tech.	0	-	-
Total	0	3/4	2

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 0	$ 9,500	$ 29,600
(b) Materials & Services	0	2,600	5,300
(c) Indirect Expenses *	0	(1%) 10,100 (2%)	21,600
Total	$ 0	$ 22,200	$ 56,500

* 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 1968

Project not in existence

12. SCOPE OF THE PROJECT

A new staff member will be recruited or a working relationship will be made with staff members from the University of California, Irvine, to pursue this project. Interrelationships among the native vegetation species are to be evaluated mathematically. Many different plant associations will be studied and the data will be useful as guidelines in interpreting the effects of ionizing radiation on desert vegetation.

13. RELATIONSHIP TO OTHER PROJECTS

This section will be correlated with all the other sections within the Division since there will be several interrelations of common interest.

14. RESULTS IN FY 1968

Project not in existence

15. EXPECTED RESULTS IN FY 1969 AND FY 1970

Data will be assembled from which ecological relationships among different plant species can be assessed mathematically. Included will be data on rainfall and temperature, plant census data from the environmental study plots, and data from soil moisture and mineral cycling studies. We expect to make progress in developing computer programs for a systems analysis approach toward understanding the desert 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:
Excited States of Molecules in Radiation Biology

3. AEC Budget Activity No.:	4. Date Prepared:
06-06-01	April - 1968
5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA
7. Person in Charge:	8. Project Term:
Richard L. Lehman	From: 1965 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2 ½	2	2
(b) Other Tech.	-	-	-
Total	2 ½	2	2

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 27,200	$ 18,700	$ 18,700
(b) Materials & Services	1,300	2,000	2,300
(c) Indirect Expenses *	(2%) 19,500	(1%) 10,100	(1%) 10,800
Total	$ 48,000	$ 30,800	$ 31,800

* 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 1968

Lehman, R. L., Fekula, O. M. and Wayland, J. R.: Sampling Bias in Scanning Proton-Recoil Tracks in Nuclear Emulsion, Nucl. Instr. and Methods (In Press)

Lehman, R. L.: The Origin of Neutron Groups in Be(α,n) Sources, Nucl. Instr. and Methods, 1968 (In Press)

Lehman, R. L.: Nuclear Design Parameters for a Monoenergetic Neutron Source, 1968 - U. S. Patent pending.

12. SCOPE OF THE PROJECT

This work is designed to advance knowledge of the electronic nature of molecules, particularly as it applies to radioprotective agents, in order to learn how certain ions and simple molecules act to protect biochemicals in solution and in protoplasm from radiolysis.

The ultraviolet charge transfer absorption bands of enzymes and other chemicals in aqueous solution will be studied and the mechanism of electronic de-excitation of primary excited species in irradiated solution will be compared for consistency with electronic state-structures based on J. W. Linnett's hypothesis of tetrahedral spin-set correlation.

The in vivo efficacy of the radioprotective chemicals selected on the basis of electronic structure considerations will be tested in the bread mold Neurospora where we will compare the ability of such chemicals to block the radiation-enhanced production of tyrosinase.

13. RELATIONSHIP TO OTHER PROJECTS

The work of L. Marton and his associates at the National Bureau of Standards on the nature of the primary energy-loss events along the tracks of fast electrons and of D. V. Cormack at the University of Saskatchewan on the exposing track energies provide the physical basis for this project. The ideas of R. S. Mulliken (Chicago University) and R. L. Platzman (Argonne National Laboratory) and the measurements of M. Kasha (University of Florida), G. L. Weissler (University of Southern California), R. D. Birkhoff (ORNL), and their students provide the background for the nature of the initial states of the excited molecules. J. W. Linnett's (Cambridge University) new ideas on valency and the electronic structure of matter serve as a basis for predicting what electronic structures are important at the chemical level. L. Augustein at Michigan State University is studying the fluorescence emissions of various materials excited by ultraviolet rays in the 1100-300 A region.

The hypothesis of A. M. Kuzin and his associates (Institute of Biological Physics, Moscow) that the ortho-quinone and ortho-phenol products of radiation-enhanced tyrosinase activity are the cause of cytological radiation damage (delay in mitosis, inhibition of DNA synthesis, altered A/T ratios in DNA, and chromosome aberrations) makes our use of this enzyme as a radiobiochemical endpoint in protection studies quite timely.

14. TECHNICAL PROGRESS IN FY 1968

The central work of the project was delayed when equipment funds for a

vacuum U. V. monochrometer were withdrawn. The occasion of this delay provided the time to complete some collaborative work in another aspect of radiological physics (see Sect. 11). Two further pieces of work, "Neutron Groups in the P_uF_4 Source", and "Random-Drift Sampling--A Study by Computer Simulation", have been completed. In the neutron group studies it was possible, by use of a new graphico-numerical method, to reconcile for the first time neutron source (integral) spectral measurements with basic (differential) beam foil measurements. The computer study established the general validity of an entirely new and rapid procedure for sampling vectors (directed line segments) in 2- and 3-dimensional space.

A new power-series solution of the Bateman equation has been derived and used as the heart of a high-speed computer program that calculates the activation and decay of isotopes subjected to a constant particle fluence. The complete history of activation and decay for the rare-earth elements has been found for 1-day activation in a 1.5×10^{12} thermal neutron fluence, as has the time course of decay of 1 g of Ra^{226} and its 17 daughters.

The time course of the radiation-induced (1-10 kR) tyrosinase activity in liquid extracts of 3-day cultures of Neurospora grown from conidia in 1/2%-sucrose-Vogel's medium has been determined by use of the DOPA $\longrightarrow$ dopachrome reaction. The enzyme activity 3 hours after irradiation of the cultures is strongly dependent on environmental factors such as oxygen and sucrose content of the culture medium, and the pH and temperature history of the cultures.

15. EXPECTED RESULTS IN FY 1969

Studies designed to test the correlation between charge-transfer absorption coefficients and radioprotective ability of certain ions and simple molecules will be resumed, and extended to include measurements in the vacuum ultra-violet by use of ultra-thin liquid layers sandwiched between special polished transmitting plates, and of thin solid films. It is expected that measurements of this type by use of the vacuum ultraviolet equipment we hope to purchase with FY 1969 funds will provide fundamental information bearing strongly on hypotheses about the nature of the primary absorption events along the paths of fast charged particles in liquids and solids.

We will continue to explore and develop radiation-sensitive biochemical end-points in Neurospora, making use of the enzyme induction methods of N. Horowitz and his associates at Cal Tech, and begin to test the efficacy of radioprotective agents in the tyrosinase system.

16. EXPECTED RESULTS IN FY 1970

Measurements with the vacuum ultraviolet spectrometer will continue and be extended to include absorption coefficients in various materials in the form of thin liquid and solid films in the 1100-1900 A wavelength region, and fluorescence/phosphorescence spectra and efficiencies of materials in this same region. The other areas of interest described for this project will be continued, with emphasis on those aspects found to be most promising this next year.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Medical Physics Instrumentation

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

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Benedict Cassen

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2 ½	2 ½	2 ½
(b) Other Tech.	-	-	-
Total	2 ½	2 ½	2 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 33,800	$ 35,200	$ 34,100
(b) Materials & Services	3,300	3,600	4,000
(c) Indirect Expenses *	(2%) 19,900	(2%) 20,100	(2%) 21,600
Total	$ 57,000	$ 58,900	$ 59,700

* 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 1968

Cassen, B. and Crandall, P.: Section Scanning with a Large Solid-Angle Collimated Detector or System of Detectors, Progress in Biomedical Engineering p. 109-114, edited by L. J. Fogel and F. W. George, Macmillan and Co., Ltd., 1967.

12. SCOPE OF THE PROJECT

The purpose of this Medical Physics Section activity is to develop new fundamental procedures and instruments that are specially applicable to currently important problems in radiobiological research and in nuclear medicine. These efforts are especially in the direction of the development of new sensors of biological information and not in the direction of rapid or more convenient data handling and processing. The new sensor developments are being used in biological and medical research applications in the Medical Physics Section under Budget Activity 06-01-01 (See Medical Physics Problems). Currently there are several major efforts in progress as well as other lesser developments.

The first, is the continuing development toward optimum performance of a high speed sectioning scanner which includes the exploration of new possibilities of appreciably increasing gamma photon utilization in radioisotope scanning. (See 14 below).

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

An efficient and greatly improved multichannel analyzer cell counting and sizing system has been developed and is being used in studying the effects of radiation and other agents in changing cell size distributions. (See 14 below).

A system is being developed to obtain short duration flash microphotographs of selected large cells emerging from a Coulter aperture and of cells subjected to stress environments. Recent improvements in holographic photography will enable an appreciable simplification and improvement of this system.

13. RELATIONSHIP TO OTHER PROJECTS

High Speed Sectioning Scanner: This phase of development and clinical testing is in cooperation with the UCLA School of Medicine Neurosurgery Group and especially with Dr. Paul Crandall of that group. Also a cooperative program with the UCLA School of Medicine Radiology Department Isotope Laboratory has been established to compare the information obtained from high speed section scans with that obtained from conventional scans on the same patients. Some others working in the field of improving radioisotope imaging are:

Mr. Hal Anger, Donner Lab., University of California, Berkeley
Mr. Robert Beck, Argonne Cancer Hospital
Dr. Merrill Bender, Roswell Memorial Inst., Buffalo, New York
Dr. David Kuhl, University of Pennsylvania

Physical Cell Measurements and Separation:

Dr. Howard Mel, Donner Lab., Berkeley, California
Dr. Marvin Van Dilla, Los Alamos Scientific Lab.

14. TECHNICAL PROGRESS IN FY 1968

High Speed Sectioning Scanner: Much clinical experience was obtained with the first model of the High Photon Utilization High Speed Section Scanner on brain tumor patients. Good stereotaxic localization results were fairly consistently obtained with administered doses of about 5 mc of technetium 99 m. A complete brain section scan is obtained in about 4 minutes and several lateral and AP sections can be obtained in a reasonable length of time. In most cases by proper setting of cut-off, a good estimate could be made of the actual size of the tumor. However, very little information could be obtained relative to the boundary configuration of the tumor, although in some patients such information, although ill-defined, was verifiable on post-mortem or after surgery. It appeared that more detailed boundary configuration obtained preoperatively could be of great value to the neurosurgeon. In order to get appreciably more resolution and to take advantage of all the lessons learned with the first scanner, a new scanner was constructed using a spherical cap nest of three-inch crystals instead of the cap of broken crystals. Each of seven three-inch crystals had its own finer grained collimator, all being lined up accurately to a common focus. This new scanner is now completed and installed in the UCLA hospital and is ready for a new patient program. Phantom tests show that the performance should be much superior to the earlier model and that much better boundary configuration information will be obtainable.

A cooperative program with the UCLA School of Medicine Department of Radiology Isotope Clinic has been established to (1) compare information obtained with high speed section brain scanning with conventional scanning on the same patients, (2) to explore the potentialities of the high speed section scanner for detecting ischemic regions in pulmonary scanning and for detecting tumors and cysts in liver scanning.

Physical Cell Measurements and Separations: Fundamental improvements have been made in cell size distribution determination methods. Reliability and reproducibility have been improved. A new preamplifier for a Coulter aperature has been developed which enables short length aperatures to be used without obtaining the Van Dilla distortion effect. The rise time of the preamplifier is less than 1 microsecond and no appreciable noise is added in the size range of blood cells.

A precision density gradient technique using gradients of bovine serum albumin was applied (a) to separate white blood cells from an overwhelming preponderance of red cells and (b) to separate leukocytes into density groups. (See Medical Physics Problems 06-01-01).

Further improvements based on lessons learned in actual use were made on a preparative electrophoretic separator.

15. EXPECTED RESULTS IN FY 1969

It is expected that still more data can be accumulated on the clinical utility of fast section scanning especially on boundary configurations of brain tumors and also on other applications such as liver and lung section scanning.

It is expected that further improvements will be made in our ability to characterize and separate viable cells without destroying their viability.

16. EXPECTED RESULTS IN FY 1970

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

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

It is expected that physical cell measurement and separation techniques can be adapted to give diagnostic information on cancer and leukemia patients.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title:
Leukemia Biology

3. AEC Budget Activity No.:
06-09-00

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Esther F. Hays, M.D.

8. Project Term:
From: 1955 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3	3 ¼	3 ½
(b) Other Tech.	1	1	1
Total	4	4 ¼	4 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 39,300	$ 46,200	$ 49,500
(b) Materials & Services	6,000	7,500	7,400
(c) Indirect Expenses *	(3%) 29,700	(3%) 30,200	(3%) 32,400
Total	$ 75,000	$ 83,900	$ 89,300

* 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 1968

Hays, Esther F., The Role of Thymus Epithelial Reticular Cells in Viral Leukemogenesis, Cancer Research, V:15: 21-26, 1968.

Hays, Esther F., Antigenetic Properties of Mouse Lymphoma Cells, Journal of Immunology - In Press, 1968.

12. SCOPE OF THE PROJECT

The Role of the Thymus in the Structural and Functional Development of Lymphoid Tissue of the Mouse.

Animals thymectomized shortly after birth or thymectomized as adults then irradiated with 800 r total body irradiation and protected with syngeneic bone marrow develop a characteristic deficit of lymphocytes in all of their lymphoid tissues, an inability to reject homografts, and an impaired agglutinin response to the inoculation of sheep red blood cells. (SRBC). Thymus grafts in these mice result in the development of a normal structural and functional status of the lymphoid system. Experiments carried out in this laboratory have shown that thymus grafts composed only of epithelial reticular cells can result in histologic and immunologic recovery of neonatally thymectomized, as well as, thymectomized-irradiated adult animals. We have found these epithelial remnant grafts to become populated with host lymphoid cells during the second week after grafting. To determine if the effect of these grafts is due principally to a humoral factor liberated by the epithelial reticular cells, or whether the presence of lymphoid cells is necessary for thymus function, they have been removed after 7 and 14 days. The recipient animals were then studied by measuring their agglutinin response to SRBC and looking at the histology of their lymphoid tissues.

Two study periods were used: 40 days, and 90 days after the grafts were removed. This was done to determine if there had been a persisting normalization of immunologic capacity and histologic appearance of the lymphoid tissues.

The Relationship of the Thymus to Lymphoma in the Mouse.

The objectives of this project are to determine the series of events that take place in the thymus prior to, and at the time of leukemic transformation. It is known that in the spontaneous lymphoma of AKR mice as well as in Gross virus induced lymphoma, the initial development of lymphoma occurs in the thymus and can be prevented by removal of this organ. We have elected to approach this problem by using thymus epithelian remnant grafts in high and low incidence leukemia strains, with and without added virus to determine the role of these cells in the development of lymphoma in the thymus. A program to evaluate the histogenesis of leukemia in the epithelial grafts by biopsy at various time intervals prior to the development of leukemia is also in progress. In this system, the ingredients are found for a predictable in vivo change to malignancy, therefore, it seems that here some of the answers to problems involving this change can be obtained.

Immunosuppression and the Development of Lymphoma in Mice.

This study is being carried out in collaboration with Donna Vredevoe, PhD., a Consultant to the Laboratory. Studies from this laboratory as well as

those of others, show that tumor-specific antigens exist in Gross virus induced lymphomas in mice. The nature of specific antigens is a matter of some doubt at present. They may represent modified transplantation antigens, entirely new cellular antigens induced and controlled by genetic materials from the virus, or changes in previously non-antigenetic cellular structures. One might suggest that if tumor antigens arise in an animal tolerant to virus antigen, but not to new cellular tumor specific antigens induced by the virus, a state of foreign antigens existing in an hostile environment would arise. In such a case it is difficult to imagine how a new antigenetic specificity can exist in the host animal unless immunologic depression which has been noted in preleukemic mice permits the development of such foreign neoplastic clones. In the case of tumor specific antigen in carcinogenic induced tumors, immunologic depression of the host has been postulated by a number of workers. Carcinogenic and mutagenic agents such as ionizing radiation and chemicals are known to lower immune responsiveness and thus might allow cell variants to establish themselves. With the advent of immunosuppressive drugs, a new area of experimentation on immune response has been opened. Although it must be recognized that such drugs are also of limited use in suppression of tumor growth, careful consideration of dosage and timing of administration can make them useful tools in the analysis of the immune response of the host to tumor cells. The recent introduction of anti-lymphocytic serum as an immunosuppressive agent adds another useful tool for study.

The design of these experiments is to study the high leukemia AKR mouse, which might be regarded as having a depressed immune response to tumor specific antigens, and a low leukemia strain mouse, C3H, which might be regarded as hypersensitive to tumor specific antigens. Pre-leukemic mice are being subjected to chronic immunosuppressive treatment to lower their resistance to foreign tumor antigens. This immune depression might be expected to promote the development of cells carrying tumor specific antigens. Both spontaneous and virus induced tumors are being studied in this manner. In summary: this study is an analysis of the effect of chronic immune depression in high and low leukemic mice, with and without injection of Gross virus.

13. RELATIONSHIP TO OTHER PROJECTS

The following lists some laboratories where research and experimental leukemia and the role of the thymus in the immune system are being carried out and whose work is of direct relationship to the studies being carried out in this laboratory.

Dr. L. Gross, Veterans Hospital, Bronx, New York
Dr. H.S. Kaplan, Stanford University, School of Medicine, Palo Alto, Calif.
Drs. Law, Maloney, Rauscher, National Cancer Institute, Bethesda, Maryland.
Dr. J.F.A.P. Miller, Walter and Eliza Hall, Institute of Medical Research, Royal Melbourne Hospital, Victoria, Australia
Dr. Britta Wahren, Institute for Cell Research, Karolinska Institute, Stockholm, Sweden.
Dr. Paul Terasaki, Department of Surgery, University of California, Los Angeles, California.
Dr. W.H. Hildemann, Department of Medical Microbiology and Immunology, University of California, Los Angeles, California.
Dr. Charles G. Craddock, Jr., Department of Medicine, University of California, Los Angeles, California.

Dr. David Imagawa, Department of Pediatrics, University of California, Los Angeles, California.

14. TECHNICAL PROGRESS IN FY 1968

The Role of the Thymus in Lymphoid Development.

I. Studies using short term thymus epithelial cell grafts: Mice of the CBA/J strain, which were thymectomized at one month of age, and six weeks of age, were lethally irradiated and protected with bone marrow cells from CBA/T6T6 mice. These animals were divided into four groups. Group 1, was thymectomized and irradiated. Group 2, was thymectomized, irradiated and grafted with a thymus epithelial remnant under the kidney capsule, removed after 7 days. Group 3, was thymectomized, irradiated, and grafted with a thymus remnant under the kidney capsule, removed at 14 days. Group 4, was thymectomized, irradiated, and the graft was left under the kidney capsule until the time of sacrifice. Some animals were killed at forty days after graft removal: and others at ninty days after graft removal. Animals which were irradiated and protected with bone marrow, but not thymectomized, showed normal histology of the lymphoid tissues and normal agglutinin responses to SRBC. Group 1: animals demonstrated depletion of the small lymphocytes in the lymphoid tissues and impaired immunologic responsiveness to SRBC. The animals in Groups 2 and 3 showed considerable variation in their immune responsiveness as well as histologic picture. The agglutinin response to SRBC was impaired in the majority of animals of both these groups. The spleen and lymph nodes showed depletion of the small lymphocytes in approximately half of the animals and in the remaining there was some evidence of repletion of these cells. Animals of Group 4 had normal immune responsiveness and tissue histology.

II. Our studies of the neonatally thymectomized animals which have had thymus epithelial cell grafts under the kidney capsules from 7-18 days, starting at 21 days of age, have shown normal responses to SRBC in 11 of 23 animals sacrificed at 90 days of age. Only 4 of these 23 animals had what could be called normal lymphoid tissues. Most of the lymphoid tissues showed depletion very similar to that found in neonatally thymectomized controls. Animals with their own thymus left in situ until 8 days of age, then thymectomized and sacrificed at 90 days of age, showed normal lymphoid tissues and immune responses. Animals thymectomized at birth and immediately grafted subcutaneously with thymus epithelial remnants which were removed at 7 days of age, showed impaired immune responsiveness, wasting and lymphoid depletion. To summarize, a weak effect of epithelial grafts in restoration of lymphoid tissues was demonstrated. It has been postulated that the thymus epithelial cells release a humoral factor which is responsible for some of the development of immunologic capacity in mice. The results of these two studies suggest that such a factor has little effect in the absence of thymus lymphoid cells.

Thymus and Leukemia.

Studies from this laboratory have shown that in vitro exposure of epithelial grafts to leukemogenic virus in leukemic transformation of these grafts. Studies of the histogenesis of leukemia in these grafts have been initiated. This is done by biopsy of virus-exposed subcutaneous grafts at various time intervals after grafting, and before the development of leukemia in them.

It has been shown that the virus infected remnant graft is populated with lymphocytes in the same way as somewhat slower than the non-virus exposed graft. It may completely resemble a normal thymus several weeks before overt leukemic transformation takes place. Animals with 5 epithelial grafts implanted have a higher incidence of leukemic transformation than those with a single graft. The preleukemic virus-exposed grafts had fewer mitotic figures in their lymphoid cells than did the non-exposed grafts. Lymphomas developed only in the virus-exposed grafts, from 9.5 to 21 weeks after they were placed in situ.

Immunosuppression and Development of Lymphoma.

Experimental diets containing azathioprine (Imuran) and 6 mercaptopurine multiple spaced dose irradiation therapy and anti-lymphocyte serum have been used as immunosuppressive agents in AKR and C3H mice with and without Gross leukemogenic virus. These studies are now in progress. The results are not yet ready for analysis.

15. EXPECTED RESULTS FY 1969.

The findings in the FY 1968 experiments of the relative ineffectiveness of the short-term thymus epithelial grafts, in thymectomized and irradiated, as well as in neonatally thymectomized mice, will lead us to perform two experiments. One will be a study of animals immediately after the grafts are removed, to see if there is a transient effect produced by the graft, which had been dissipated at the time of study, (2-3 months after grafting). Secondly, since Miller (Clin. Expt. Immunol., 1: 61, 1966) has reported, restoration on neonatally thymectomized mice bearing syngeneic thymus implants for one week, we will study whole thymus grafts in our system. If it is shown that short-term whole thymus grafts restore animals in a situation where epithelial grafts cannot, this will constitute experimental evidence to postulate a definite role for the donor thymus cortical lymphoid cells. Donor cells have been shown to be present one week after grafting and then to be completely replaced by host lymphoid cells. Most of the cortical lymphocytes which are known to be actively proliferating cells, with a life span of three days, are believed to be destroyed within the thymus. The experiments proposed here might provide an answer to the purpose of this intense thymus lymphocyte activity, i.e., that the intra-thymic lymphocyte destruction, possibly mediated by epithelial reticular cells, releases products which are responsible for the restoration of neonatally thymectomized, and thymectomized-irradiated mice.

A direct relationship between the presence of the thymus and the persistence of lymphoid cells in the bone marrow has been shown by our studies. Neonatally thymectomized mice show a progressive loss of bone marrow lymphocytes, from normal levels at birth to their virtual absence in wasted animals to 8 weeks of age, indicating that the maintenance of these cells is controlled in some way by the thymus. Recent studies have demonstrated that inoculations of adult bone marrow cells into neonatally thymectomized mice, result in protection from the intestinal illness which produces the characteristic wasting syndrome. Immunologic capacity and histologic appearance of the lymphoid tissues, however, were not restored to normal. This is similar to the appearance of thymectomized irradiated adults which have been protected with adult bone marrow. The Peyer's patches in both of these animal preparations have been found to be histologically normal. Therefore, further

studies to clarify this interesting finding will be done. Investigations of the possibility as to whether the bone marrow lymphoid cells are responsible for direct population of the Peyer's patches, making the mechanism of development of this system for intestinal immunity somewhat different than that which is known to be present in the other lymphoid tissues such as spleen and lymph nodes, will be carried out.

Studies will also be undertaken in FY 1969 to determine the role of irradiated thymus remnants in leukemogenesis using technics similar to those of our previous studies. We will look at remnants given 800 r and 5000 r before and after exposure to virus.

Studies concerning the immunologic status of pre-leukemic mice using the response of these animals to different antigens as a measure of the immunosuppression on leukemogenesis will be continued.

16. EXPECTED RESULTS FY 1970

The interaction of thymus and bone marrow on lymphoid tissue and immune response will be continued object of studies in both normal and leukemic or pre-leukemic mice.

The question of the exact mechanism of the leukemic transformation in the thymus medullary cell, virus, thymus, lymphoid cell system will be approached. One consideration will be whether transformation results from an interaction of RNA of the epithelial reticular cells in conjunction with virus, on the leukemia susceptible lymphoid cells. The effects of ionizing radiation in this system will also be object of further experiments. Studies are planned to irradiate the medullary cells, lymphoid cells, and virus and to observe the effects of this treatment on the development of leukemia in susceptible as well as resistant hosts.

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 - 1968

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

6. Working Location:
UCLA and Harbor General Hospital

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

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	4 3/4	5 1/2	5 1/2
(b) Other Tech.	1/2	1/2	1/2
Total	5 1/4	6	6

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 66,900	$ 76,300	$ 78,700
(b) Materials & Services	17,000	16,800	17,600
(c) Indirect Expenses *	(5%) 49,600	(6%) 60,400	(5%) 54,000
Total	$ 133,500	$ 153,500	$ 150,300

* 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 1968

Taplin, G.V., Swanson, L.A., Hayes, M. Recent Advances In Kidney Scanning. Lahey Clinic Bulletin, 16: 361-372, 1967.

Taplin, G.V., Poe, N.D., Dore, E.K., Greenberg, A. Bronchial Patency and Aerated Space Assessment by Scintiscanning. Lahey Clinic Bulletin, 16: 297-312, 1967.

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Webber, M.M., Bloomer, W.E., Crandall, P.H., Drainkard, J., Glober, G., Herbst, H.H., Johnson, D.E., Killeffer, F.A., McAlpin, R.N., Pope, M.A., Stern, W.E., Swanson, L., Taplin, G.V., Weidner, W.A., UCLA Interdepartmental Conference: The Use of Radioisotope Scanning in Medical Diagnosis: Applications in Diseases of the Brain, Lung, Liver and Heart. Annals of Internal Medicine, 67: 1059-1083, November, 1967.

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Taplin, G.V., Poe, N.D., Swanson, L.A., Dore, E.K., Greenberg, A. Scintiscanning in the Assessment of Regional Pulmonary Function, Chapter 30 of Clinical Cardiopulmonary Physiology, Third Edition, Grune-Stratton, N.Y. (In press).

Taplin, G.V. The Scintillation Scan, Clinical Scintillation Scanning, Chapter 5, Hoeber Medical Division, Harper & Row, publishers (In press).

Swanson, L.A., Hayes, M., Kennady, J.C., and Taplin, G.V. A Ten Probe Rapid Scanner - Preliminary Evaluation, Nuclear Medicine Symposium, Lahey Clinic, Boston, Mass. Sept. 1967.(Scientific Exhibit)

Swanson, L.A., Hayes, M., and Taplin, G.V. Preliminary Experience With A Dynapix, 53rd Scientific Assembly and Annual Meeting of the Radiological Society of North America, Inc., Chicago, Ill. Nov.26-Dec. 1, 1967. (Scientific Exhibit)

Kennady, J.C., and Taplin, G.V. Assessment of the Cerebral Microcirculation II, American Coll. of Surgeons, Chicago, Ill. Oct. 2-6, 196/.(Sc. Exhibit)

Ibid. International College of Surgeons, Las Vegas, Nev. Nov. 1967 (Scientific Exhibit).

Poe, N.D., Dore, E.K., Swanson, L.A., and Taplin, G.V. Proper Interpretation of Lung Scans in Diagnosing Pulmonary Embolism. Am. J. Card. 21: 113, 1968.

12. SCOPE OF THE PROJECT

The major goal is to extend the application of radioisotopes in medical diagnosis. New tracer procedures should reveal reliable information which is otherwise impossible, impractical or hazardous to obtain or provide supplementary data which aid evaluation of other diagnostic procedures. Scintillation counting and organ imaging techniques are unique in that they may be performed externally to the body and permit measurement of tracer as it enters or leaves internal organs. Organ size, shape and position may be visualized by scintiscanning and abnormalities such as tumors, cysts or abscesses may be detected as areas of either increased or decreased tracer concentration. External monitoring techniques and dynamic scintiphotography permit assessment of organ blood flow with relative simplicity.

Presently, the clinical investigations include studies of organ function, structure and hemodynamics in conjunction with standard diagnostic procedures. Patients with diseases of the RES, kidneys, liver, heart, lung, brain, bone and placenta are being investigated. Parallel studies are made in animals. T his dual approach has led to the development of several valuable radioisotope procedures in the past and to their continuing improvement. New developments during the past year include sequential kidney and liver scanning procedures, the development of technetium 99m and Indium 113 m chelates for dynamic kidney scanning, further improvement in the techniques for preparing suspensions of radio-albumin aggregates of narrow size range distribution in three main sizes for specific purposes. Microaggregates of 1-5μ for simultaneous liver and spleen scanning and for measuring liver blood flow and phagocytic capacity of the RES. Suspensions of a mean size of about 15μ labeled with technetium 99m for brain hemisphere scanning and larger size suspensions (mean about 30μ) for lung scanning with rapid imaging devices. The latter two preparations have the added feature of being more rapidly cleared from the brain and lungs respectively because of their unique property of relative fragility as compared with iodinated aggregates of the same size. This unique feature of the technetium labeled albumin aggregates will be further exploited in the future for measuring regional blood flow of various organs or areas of the body which can be injected via an arterial catheter.

With the availability of the Ter Pogossian x-ray image amplifier scintillation camera during the past year, a major advance in dynamic brain scanning has been accomplished. This device has made possible the visualization of tracer traversal through the cerebral microcirculation by providing clear images at 0.2 second intervals. Preliminary findings indicate that dynamic scintiphotography is a valuable supplement to

cerebral angiography and helps distinguish neoplastic from vascular lesions. The rapid diffusibility of xenon 133 into tumor regions becomes distinctly apparent within a few seconds whereas agents which remain in the vascular system such as 5μ size microaggregates of albumin show greater concentration in surrounding regions during this time. Similar camera studies of cardiopulmonary hemodynamics show promise as a means for detecting intracardiac shunts and other abnormalities of cardiopulmonary hemodynamics. Similar renal studies were far less promising with intravenous injections however the same techniques used in brain hemisphere scanning appear to be almost equally applicable in studying blood flow abnormalities within a single kidney following renal artery injection of tracer materials.

Considerable progress has been made in the past year in developing closer coordination of the nuclear medicine division of the laboratory with that in the medical center and Harbor Hospital. Plans are underway to move the nuclear medicine facilities in the laboratory to the biophysics area in the medical center which is adjacent to radiology and the vivarium. This amalgamation of the radioisotope divisions consolidates the clinical and basic nuclear medicine research and radiopharmaceutical development in a single location within the medical center. This arrangement has greater potentiality for making rapid advances in nuclear medicine in the future.

13. RELATIONSHIP TO OTHER PROJECTS

Similar clinical studies are being made at most of the major universities and medical centers in the United States; in various foreign medical centers such as the University of Lund, Malmo, Sweden; University of Heidelberg, West Germany; University of Athens, Greece; University of Pisa, Italy; Institute of Cancer Therapy, Lisbon, Portugal; Institute of Cardiology and Neurology, Mexico City; Institute for Nuclear Studies, San Paulo, Brazil; 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 French Atomic Energy Agency at Dorsay.

14. TECHNICAL PROGRESS IN FY 1968

Lung Scintiscanning Studies: Radioaerosol inhalation and perfusion type lung scanning procedures have been used in conjunction with radiography, angiography and conventional pulmonary function testing in further investigation of pulmonary embolism, tuberculosis, emphysema, bronchogenic carcinoma and other obstructive bronchopulmonary diseases to better define the physiological significance and diagnostic usefulness of the scintiscanning procedures in the management of pulmonary disease. The 10 probe rectilinear scanner has facilitated these studies by greatly reducing scanning time while giving lung images of higher resolution. Preliminary clinical studies with xenon gas inhalation compared with radioaerosol lung retention indicates indicates that the two procedures give similar information on regional ventilation in normal subjects and patients without obstructive bronchopulmonary disease. In the latter, delayed scans performed 6-18 hours following aerosol inhalation are needed to obtain reliable information on regional ventilatory disturbances. The combined inhalation perfusion scanning procedure which can now be performed in all four views with each agent in less than ninety minutes

has proved especially useful in distinguishing pulmonary embolism from from regional bullous emphysema.

Renal Studies: Iothalamate and orthoiodohippurate renography and rapid sequence kidney scanning has been continued in studying patients with unilateral renal vascular hypertension and in upper urinary tract disorders. The dynamic kidney scanning with the 10 probe rectilinear scanner has been demonstrated to be a real advance in kidney scanning and in differential kidney function assessment. The scan images of the kidneys at two minute intervals help locate the site and nature of intrarenal abnormalities of tracer transport kinetics. The procedure helps distinguish unilateral partial upper urinary tract obstruction from unilateral renal vascular ischemia. In the former tracer transport through the kidney may be slowed but tracer accumulates in the pelvis. The renogram may show identical patterns in both conditions. In polycystic disease of the kidneys the dynamic scanning procedure readily demonstrates the sites and degree of intrarenal obstruction which explain the delayed drainage phase of the renogram in this disorder. Another useful application of sequential scanning with radiohippuran is in the visualization of the kidneys in patients with severe azotemia. Nearly all patients studied to date in whom the kidneys were not visualized by conventional intravenous pyelography they become readily detectible within the first half hour by sequential scanning. Preliminary clinical trials with technetium 99m and Indium 113m chelates have been initiated and results with these short lived nuclides appear most promising as test agents for the assessment of differential glomerular filtration. Simultaneous measurements of GFR and estimated renal plasma flow (ERPF) have been made in numerous patients simultaneously with the sequential scanning procedure. The single injection technique and compartmental analysis of the blood disappearance curves appear to give useful clinical information but so far results are somewhat at variance with those obtained by the conventional constant infusion technique.

Liver and RES Studies: Beginning July 1967, Doctor Hideo Yamada of the University of Tokyo joined our group to work specifically in this area. He has conducted basic studies in animals to show the relation of particle size of radiocolloids to liver extraction efficiency during their first pass through the organ in rats. He has demonstrated that extraction efficiency increases from 60-70% with small colloids (10-20μ) to 90-95% with radioalbumin microaggregates (1-5μ). In clinical studies with the same size ranges splenic uptake and visualization increases directly in proportion to mean size of the colloidal suspension. The suspension of microaggregates (1-5μ) appears to be a superior liver-spleen scanning agent than colloidal gold or small colloidal radioalbumin. Preliminary sequential liver and upper abdominal scans using radio rose bengal and the ten probe scanner show considerable promise for this procedure as a means for differentiating medical versus surgical jaundice. Partial extra hepatic biliary obstruction is readily recognizable by demonstrating delayed tracer entry into the intestine and excessive accumulation in the major bile ducts both intra and extra hepatic. Complete biliary obstruction of intra or extra hepatic origin remains difficult to distinguish. Much further experience is needed to assess the role of sequential liver-upper abdominal scanning as a means for making this differentiation.

During liver-spleen scanning studies of cirrhotic patients, using the microaggregates of albumin, an interesting observation has been made repeatedly. The bone marrow in the spine, pelvis, sternum and ribs

has been visualized in cases of severe hepatic failure just as well with the 1-5μ size aggregates as with colloidal gold and small radio-albumin colloids of 10-20mμ size. Furthermore, the retention in the liver spleen and bone marrow is several times longer for the microaggregates versus the small organic radiocolloids. This feature of the microaggregate suspension is a definite advantage in conventional liver spleen scanning and may provide a practical means for estimating differences in digestive function of the RE cells in each of the three main organs by sequential scanning.

Tomographic Capacity of the Ten Probe Rectilinear Scanner: When this device is operated with the short focus (3.5 inch) high resolution collimator, it has a definite tomographic capacity. This unique feature has been best demonstrated in brain scanning where it has been shown to be capable of visualizing the choroid plexuses in the posterior horns of the lateral ventricles and by better resolving the size and location of ill defined routine scan lesions. The practical value of this tomographic capacity appears less rewarding for better visualization of radio negative lesions such as those in the liver and lung.

Preliminary Evaluation of the Ter Pogossian Camera: This rapid imaging device has been shown to have its best application in brain hemisphere scanning using Xenon-133 and 99m Tc as the test agents in 2-10 mc doses by internal carotid artery injection. Its use in studying cardiopulmonary hemodynamics is promising but much less so than for brain blood flow studies. Its limited field of view precludes its application for hemodynamic studies of both kidneys simultaneously. However, it has potential value as a supplement to selective renal arteriography in studying one kidney separately.

Phantom Studies: A three dimensional phantom has been devised to compare the imaging capacity of various scanners and camera type devices. It consists of hollow or solid rods of three different sizes running diagonally through a tank so that an instrument's imaging capacity may be determined simultaneously at increasing depths, with a single scan examination. It is a modification of a phantom originated by G.J. Hine who used a line source running diagonally from top to bottom of a box shaped container.

Radioactive tubes in the new phantom were scanned in air and in water. Inactive solid rods were scanned in a background of radioactive water. Comparative studies with 99m Tc were made with conventional scanners having 3 and 5 inch diameter crystal detectors with focus collimators, a 5 inch dual probe scanner, a 10 probe rectilineat scanner, an Anger camera and a Ter Pogossian x-ray image amplifier camera. Preliminary results indicate that the three dimensional phantom is superior to point or line sources and to single plane phantoms. It is simpler to use yet more versatile than organ phantoms in that an instrument's capacity to image simulated lesions at increasing depths from the surface may be determined by a single scan examination. The physician can readily appreciate differences between instruments by simple scan comparison. More complicated analytical methods are seldom needed for clinical purposes.

15. EXPECTED RESULTS IN FY 1969

Lung Studies: Combined radioaerosol inhalation and perfusion type scintiscanning will be continued to better establish their role as supplemental procedures in the diagnosis and management of pulmonary embolism, tuberculosis and chronic obstructive bronchopulmonary disease. Greater emphasis will be placed on the application of these two scintiscanning procedures in the diagnosis and management of bronchogenic carcinoma and related obstructive pulmonary disorders. In addition, with the acquisition of an improved Anger type scintillation camera with wider field of view and with the full time services of Doctor Isawa from Sendai, Japan as a Dernham Foundation Senior Fellow in Oncology, plans are underway to initiate radioactive gas studies as a third radioisotope parameter in the assessment of pulmonary function. The three types of scintiscanning procedures will be coordinated with the pulmonary function section of the Department of Medicine under Doctor Wasserman at Harbor Hospital. This coordinated approach promises to be fruitful in gaining a better understanding of the disturbed pulmonary physiology in obstructive lung disease and in evaluating the potential contributions of the radioisotope procedures in the diagnosis and medical and surgical management of such patients.

Renal Studies: With the availability of the improved Anger camera plus the ten probe rapid scanner, dynamic kidney scanning will be continued and advanced by using the new kidney specific agents labeled with 99m Tc and Indium-113m developed in the past year. Emphasis will be placed on quantitation of the sequential scan data to devise practical methods for assessing differential kidney function which could replace urologic methods requiring bilateral ureteral catheterization. The dynamic kidney scanning will be coordinated with the single injection type clearance methods for estimating renal plasma flow and glomerular filtration rates. The radioisotope studies will be made in cooperation with the nephrology section of the Department of Medicine and the Urology division of the Department of Surgery. It is anticipated that this cooperative effort will lead to the development of improved methods of kidney function assessment with radioisotopes which are sorely needed in the management of unilateral renal vascular disease with hypertension and in obstructive uropathies.

Liver and RES function studies: Sequential liver and upper abdominal scanning with radio rose bengal and radiocolloids will be continued in an effort to make progress in solving the difficult diagnostic problem of distinguishing medical from surgical jaundice. Results of the scanning procedures will be correlated with cholangiography, liver angiography and with results of biochemical tests of liver function.

RES function studies will include serial scan examinations of the liver, spleen and bone marrow in cirrhosis and in patients with hypersplenism using 1-5μ microaggregates of radioalbumin. Such studies on the relative rates of tracer removal from these organs should provide valuable and potentially diagnostic information on the proteolytic digestive capacity of the RE cells in each of these organs. One goal is to develop a practical clinical test for hypersplenism. Another goal of the liver spleen scanning work is to devise means for improving the resolution of liver lesions. Currently, conventional scanning procedures miss at least 25-30% of small metastatic lesions.

Evaluation of New Organ Imaging Devices: Comparative phantom and clinical studies will be made to determine the relative advantages and limitations of conventional scanners and camera type imaging devices, using the three dimensional phantom and radionuclides of different energies measured in air, water and tissue equivalent scattering media. The new Anger type scintillation camera will be compared with the Ter Pogossian camera in dynamic studies of blood flow in the brain, heart, lung, liver and kidney.

16. EXPECTED RESULTS IN FY 1970

Much of the clinical investigative work during this year will consist of the application of new short lived technetium and Indium labeled radiopharmaceutical agents in the development of dynamic scanning procedures as a continuation of the work described for the previous year. Additional applications of radionuclides in medicine include the development of scanning procedures for the detection of intestinal obstruction. Conventional radiography with barium sulfate could be improved by substituting insoluble radionuclide preparations in minute quantities. Efforts in this direction will be made using radio rose bengal and radio colloids of Indium 113m and Tc 99m. Further medical applications of the whole body counter facility include the development of methods by which kidney function may be measured in infants and young children using minute quantities of short lived kidney specific test agents such as the chelates of Indium-113m and Tc 99m.

Other new endeavors will include regional blood flow studies of the myocardium, liver, kidney and other organs using the improved Anger camera and 99m Tc albumin 10-25μ macroaggregates. The high fragility and transitory retention of this agent should minimize the slight potential hazard to levels far below those of routine contrast agents and angiography.

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 - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Norman D. Poe, M.D.

8. Project Term:
From: 1958 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3 1/4	2 3/4	2 3/4
(b) Other Tech.	-	1/2	1/2
Total	3 1/4	3 1/4	3 1/4

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 28,100	$ 31,100	$ 31,300
(b) Materials & Services	6,600	7,600	10,100
(c) Indirect Expenses *	(2%) 19,900	(2%) 20,100	(2%) 21,600
Total	$ 54,600	$ 58,800	$ 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 1968

Poe, N.D., Swanson, L.A., and Taplin, G.V. Physiological Factors Affecting Lung Scan Interpretation. Radiology, 89:661-666, Oct. 1967.

Taplin, G.V., Poe, N.D., Dore, E.K., and Greenberg, A. Bronchial Patency and Aerated Space Assessment by Scintiscanning. Lahey Clinic Bulletin, 16: 297-312, 1967.

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Poe, N.D., Dore, E.K., Swanson, L.A., and Taplin, G.V. Proper Interpretation of Lung Scans in Diagnosing Pulmonary Embolism, Am. J. Card.21:113,1968.

12. SCOPE OF THE PROJECT

This Section continues to be the testing arm of the Clinical Nuclear Medicine and Radiodiagnostic Agent Development Sections. New procedures and radiopharmaceutical agents are first tested in animals to determine toxicity and physiological features. Then experimental models are prepared surgically, pharmacologically or by other means to produce pathological conditions simulating various disease states in man. The parameters measured by a new procedure are compared with accepted ones such as roentgenograms, cardiac catheterization data, blood flow measurements, arterial gases and pH values, electrocardiograms, autoradiograms and various in vitro analyses to determine its validity and safety for clinical application.

With the controlled conditions obtainable in the laboratory, new as well as established radioisotope procedures are employed to study basic physiological problems. For example, long-term studies of pulmonary arterial perfusion changes in atelectatic lungs are possible with serial lung perfusion scanning.

In a like manner acute and chronic ventilation changes following pulmonary arterial occlusion can be determined by inhalation scanning. The latter technique also provides a simple and effective biological model for studying the distribution and clearance of inhaled aerosols with potentially wide application outside the nuclear medicine field.

In addition, it is also within the scope of this project to provide radioisotope equipment and animal facilities for related or joint projects elsewhere in the Laboratory and the UCLA Medical School.

13. RELATIONSHIP TO OTHER PROJECTS

The activities in this section are related to projects being carried out at the UCLA Medical School (Departments of Surgery and Urology), and the Nuclear Medicine Division at Harbor General Hospital, Memorial Hospital of Long Beach and the Los Angeles County Olive View Hospital. Our inhalation lung scanning studies are related to similar work by Doctor Felix Pircher, Houston, Texas. Studies of pulmonary emboli are being performed by Doctor Henry Wagner, Baltimore and Doctor Wil Nelp, Seattle. Doctor Lester Bryant, University of Kentucky is using scanning for studying blood flow changes in bronchial obstruction.

14. TECHNICAL PROGRESS IN FY 1968

Aerosol Clearance From the Lower Respiratory Tract: Using inhaled gold-198 colloid, it was determined that this material disappears from the lung in a biphasic manner. A first rapid phase is completed in 6-12 hours and appears to represent material removed by the ciliary escalator mechanism. An accentuated, first phase is seen in normal subjects with rapid, irregular respiration and in patients with severe obstructive disease. Scans show that the aerosol is concentrated in the major bronchi. In patients with bronchiectasis small deposits are seen in the smaller bronchi presumably at sites of partial obstruction. With further studies of bronchial distribution and clearance, it is anticipated that this technique may give information analogous to that obtained from bronchography but more safely.

A slow clearance phase ($T\ 1/2 > 24$ hours) follows the initial rapid phase. However, in normal subjects or intubated animals, only the second phase may be evident. Alveolar retention of inhaled aerosol is proportional to isotope concentration in the lung following a single breath of Xenon-133 gas. Therefore, the alveolar distribution of aerosol is an index of ventilation. In most cases of parenchymal lung disease, scans performed soon after the aerosol inhalation give a satisfactory ventilation picture. However, if excessive bronchial deposition is present, a delayed scan should be obtained to evaluate regional ventilation.

Diagnosis of Pulmonary Embolism With Combined Perfusion-Inhalation Lung Scanning: A major problem in the diagnosis of pulmonary embolism is the inability to differentiate pulmonary ischemia of embolic origin from that produced by other forms of lung disease. In spite of compensatory mechanisms lung made ischemic by emboli remains ventilated to varying degrees. This has been confirmed by radiogas and radioaerosol inhalation studies, in animals with experimental emboli and surgical pulmonary arterial occlusion. Preliminary clinical data with 99m Tc-albumin aerosols and 133 Xe gas show good correlation and indicate

that there is a slight decrease in ventilation immediately after vascular occlusion with a greater decrease developing over a few weeks. Complete absence of ventilation or atelectasis has not been observed.

Limitations of Quantitative Lung Scanning: Because routine rectilinear scanning equipment cannot display data in three dimensions, quantitative lung scanning is not possible. The practical limits of quantitation have been determined by phantom studies and experiments in animals following postural changes and bronchial obstruction. If blood flow changes are homogeneous and the regions in question are well separated, eg. separate lungs, quantitation of relative flow between the two regions is possible with a high degree of accuracy, when compared with direct counting of excised lungs. However, regions within one lung cannot be adequately separated and therefore flows cannot be quantitated. Nevertheless, by maintaining the same geometry in repeated scan examinations, changes in flow in small regions of an individual lung can be detected and estimated in a semiquantitative fashion. While this has some clinical usefulness, it has proven particularly valuable experimentally for following regional blood flow changes in a physiological manner.

Drug Treatment of Hypotension in Pulmonary Embolism: Clinically, norepinephrine is commonly used to treat hypotension following pulmonary embolism. This agent is a potent pulmonary arterial vasoconstrictor and therefore might actually be contraindicated as a therapeutic agent for massive pulmonary embolism, which is associated with pulmonary hypertension. Comparisons of isoproterenol (cardiotonic but not vasoconstrictive) and norepinephrine following experiment of pulmonary embolism and shock, showed that isoproterenol increased cardiac output while norepinephrine raised the pulmonary arterial pressure and usually reduced cardiac output. It is concluded that isoproterenol should be explored clinically in the treatment of pulmonary embolism.

Lung Scan - Autopsy Correlations: The results of autopsies and scans in 80 patients who died of various causes at intervals ranging from weeks to several months following lung scans showed good correlation in most cases of pulmonary embolism and localized parenchymal diseases eg. tumors, pneumonia, bullae and pleural effusion. Poor correlation was found with rapidly changing conditions such as pulmonary congestion and edema and also in many patients with focal emphysematous disease.

Proper Interpretation of Lung Scans: Considerable effort has been expended to determine the physiological and pathological conditions that influence lung perfusion and thereby alter lung scan images. The main purpose of this study is to identify the nonembolic causes of regional pulmonary ischemia which may simulate pulmonary embolism. Regional pulmonary ischemia can be classified into three major categories: Physiological, including normal variants, postural change and secondary responses to congestive heart failure; pathological with abnormal chest roentgenographs as found in pneumonitis, pleural effusions and tumors; and pathological with negative chest roentgenographs specifically certain stages of emphysema and asthma as well as embolism itself. These variables have been defined to stress the importance of the history, physical and chest x-ray when using the lung scan to support a diagnosis of pulmonary embolism.

15. EXPECTED RESULTS IN FY 69

Ciliary Clearance in Transplanted Animal and Human Lungs: In conjunction with the Department of Surgery at UCLA, bronchial clearance rates in normal and transplanted lungs will be studied following the inhalation of coarse (5-10μ) radioaerosols. Transplanted lungs ventilate adequately but are prone to infection. It is possible that impaired ciliary activity may be a major or contributing factor.

Course of Pulmonary Embolism: Our preliminary serial lung scanning studies in pulmonary embolism will be expanded to verify our initial impressions on the rates of resolution and blood flow restoration in this disease. Two other groups have shown divergent results and these differences must be resolved. Our data indicate that uncomplicated major emboli resolve rapidly but that recovery is much slower and less complete if other cardiopulmonary disorders are present. This differentiation has not been emphasized by others.

Evaluation of Combined Lung Scans in Relation to Other Pulmonary Function Tests: To determine the supplementary or complementary value of inhalation and perfusion scanning to routine pulmonary function tests, combined scans will be performed immediately after complete pulmonary function tests in patients with a variety of pulmonary diseases. Since many of these patients will be treated surgically, pathological correlation will also be possible. This study also will aid materially in gaining a better understanding of both types of lung scans and their diagnostic significance.

Factors Influencing Flow Changes in Unilateral Pulmonary Hypoxia: A completed study has shown that pulmonary blood flow changes (measured by serial lung scanning) in the ventilated and non-ventilated lung become fixed 2-4 months after bronchial occlusion. Previously, it was assumed these findings were due to changes in the anoxic lung, but analysis of the data suggest that vascular changes occur in both lungs. A project is now underway to localize the source of these changes.

16. EXPECTED RESULTS IN FY 1970

Work will continue towards better understanding of basic pulmonary physiology using isotopic methods by further sophistication of present techniques and use of new ones. The latter will include dynamic studies with scintillation cameras. Radioactive gases and highly soluble aerosols will permit dynamic studies not possible with present lung scanning agents. This work is expected to provide one or more new parameters in the assessment of pulmonary function.

To complement pulmonary studies, cardiac scanning will be re-explored. Previous experiments with rubidium-86 and work of others have not been too successful but more suitable agents are becoming available and with improved scintillation cameras, studies of regional myocardial ischemia now show considerable promise.

Inhalation lung scanning techniques will be improved to shorten aerosol administration time and to increase the percent retention of the inhaled

material. While this procedure may be replaced by gas methods for estimating regional ventilation, it has considerable potential value as a useful supplement to bronchography; for studying the bronchociliary mechanism and also for determining the distribution pattern of medicinal aerosols. It is further anticipated that work will be directed to better understanding of aerosol deposition within the lungs. In this regard, joint projects with bioengineering and/or public health groups are being explored.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Hemodynamics

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA and Harbor General Hospital

7. Person in Charge:
John C. Kennady, M.D.

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	3 1/2	2 1/4	2
(b) Other Tech.	1/2	1/2	1/2
Total	4	2 3/4	2 1/2

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 39,300	$ 26,700	$ 24,600
(b) Materials & Services	8,300	8,500	10,300
(c) Indirect Expenses *	(3%) 29,800	(2%) 20,100	(2%) 21,600
Total	$ 77,400	$ 55,300	$ 56,500

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

11. PUBLICATIONS IN FY 1968

Kennady, J.C., and Taplin, G.V. Shunting in the Cerebral Microcirculation. Amer. Surg. 33: 763-771, 1967.

Kennady, J.C. Conventional Diagnostic Procedures and Pathophysiological Consideration. Proceedings of Symposium on Nuclear Medicine - Its Current Status In Medical Practice, Sept. 1967, USAEC Div. of Tech. Inf.(In press).

Kennady, J.C., and Taplin, G.V. Regional Cortical Blood Flow Studies With Radioalbumin Macroaggregates. Proceedings of Conference on Blood Flow Through Organs and Tissues. Univ. of Glasgow, Scotland, March 1967, Publishers, Messrs. E & S Livingston Ltd.

Kennady, J.C., and Taplin, G.V. Assessment of the Cerebral Microcirculation II, Amer. Coll. of Surg. Chicago, Ill. Oct. 1967, Scientific Exhibit.

Kennady, J.C., and Taplin, G.V. Assessment of the Cerebral Microcirculation II, Int. Coll. of Surg. Las Vegas, Nev. Nov. 1967.

Swanson, L.A., Hayes, M., Kennady, J.C., and Taplin, G.V. A Ten Probe Rapid Scanner - Preliminary Evaluation. Scientific Exhibit at Symposium on the Clinical Applications of Nuclear Medicine, Lahey Clinic, Boston, Mass. Sept. 1967.

Swanson, L.A., Hayes, M., Kennady, J.C., and Taplin, G.V. A Ten Probe Rapid Scanner - Preliminary Evaluation. Scientific Exhibit at 53rd Scientific Assemly and Annual Meeting of the Radiological Society of North America, Inc., Chicago, Ill. Nov. 26-Dec. 1, 1967.

Knox, R., Batzdorf, U., and Kennady, J.C. Radioactive Uptake by Human Brain Tumors in Tissue Culture. Clin. Res. 16, 1968 (abstract).

Kennady, J.C., Swanson, L.A., and Taplin, G.V. Assessment of Cerebral Blood Flow with the Magnacamera. Clin. Res. 16, 1968 (abstract).

Kennady, J.C., Potter, R., Chin, F., and Swanson, L.A. Radioisotope Scintiangiophotography of Cerebral Hemisphere Lesions. J. Nuc. Med. 9: 1968 (abstract).

Kennady, J.C., and Taplin, G.V. Assessment of the Cerebral Microcirculation III. Amer. Assoc. of Neurological Surgeons, Chicago, Ill. April, 1968, (Scientific Exhibit).

Knox, R., Batzdorf, U., and Kennady, J.C. Human Brain Tumor Cell Affinity for Radioisotopes. J. Nuc. Med. 9, 1968 (Abstract).

12. SCOPE OF THE PROJECT

This Section is now well advanced in the realm of Basic Clinical Nuclear Medicine. Its objective to develop improved tracer techniques for the detection and evaluation of abnormal hemodynamics, particularly of the brain, has extended to elucidation of the microcirculation. Externally performed scintillation counting methods have the distinct advantage of simplicity, safety and of testing under physiological conditions.

Angiography and other radiographic and neurosurgical procedures used to evaluate vascular lesions, are far more complicated and involve definite hazards. Radiographic procedures give morphological information of diagnostic value in respect to the macrocirculation. Photoscanning using various test agents not only provides information regarding blood flow distribution in the macrocirculation but also in the microcirculation. Hence the tracer procedures give valuable supplementary information in respect to underlying physiological disturbances at the cellular level.

The research activities of this section during this past year have been concerned with the assessment of cerebral hemodynamics with 99m Tc labeled albumin macroaggregates (10-50μ size) and microaggregates (1-8μ size), 133 Xenon in saline and 99m Tc pertechnetate. The rapid passage of the last three test agents necessitates the use of a scintillation camera. The goals of the Section are to develop reliable external monitoring procedures for evaluating abnormalities of the microcirculation.

Progress in microneurovascular surgery has made great advances in reopening occluded arteries and re-establishing blood flow to the impaired microcirculation of many disabled patients followed by subsequent improvement. Hence a nontraumatic method for assessment of relative flow in the microcirculation prior and following operative procedures of the brain is of prime importance.

This Section has also underway studies of the comparative uptake of radioisotopes by different human brain tumor cell cultures. The objectives are to determine whether there is a passive or active transport of the tracer into the tumor cells, the role of the blood-brain barrier in radiolabeling the tumor cells and what types of test agents are best used for the external detection of specific tumor types. The goals therefore are to develop test agents with specific tumor type affinity to not only detect small tumors by the brain scanning technique but ascertain the tumor type prior to surgery.

13. RELATION TO OTHER PROJECTS

Studies in measurement of cerebral blood flow are being conducted by the Wadsworth VA Hospital in Los Angeles by Doctors Oldendorf and Cassen; at Duke University by Doctors Odom and Tindall; in England by Doctors Harper and Jennett; in Sweden and Denmark by Doctors Ingvar, Hoedt Rassmussen and Lassen.

Studies on brain tumor culture and implantation using radioisotopes are being conducted at the University of Kentucky by Doctors Wilson and Norrell; at Duke University by Doctor Maholey; in Canada by Doctors Tator and Morley.

14. TECHNICAL PROGRESS IN FY 1968

During the fourth year of basic studies in primates the minimum cerebral toxicity level of 131 I labeled albumin macroaggregates (RAMA) 10-50μ size was established (2 mg carrier albumin). Approval for trial in selected patients was granted by the committees in human use of radioisotopes both at UCLA Center for the Health Sciences and the Los Angeles County Harbor General Hospital. With RAMA suspensions in the 15-45μ size range, the estimated minimum toxic dose in normal man (extrapolated from the toxicity data in monkeys) is approximately 500 times larger than the dose required

for scanning. Brain hemisphere scanning in selected patients with primary or secondary brain tumors is in progress. The EEG and neurological examinations done prior to, during and following RAMA injections to date have revealed no untoward neurological sequelae. By contrast, areas of increased radiodensity in 99m Tc scans appear 'cold' in RAMA scans. This indicates an impaired microcirculation within the tumor. If current successful results continue, patients with cerebrovascular disorders will be assessed in the near future.

The Ter Pogossian scintillation camera permits continuous visualization of the entire cerebral hemisphere and dynamic examination of the passage of radioisotope tracer through the cerebral circulation. Data, taken from the television monitor, is permanently recorded on video tape for playback on polaroid film as pictures of a single, 1/30th of a second frame or of integrated frames for any desired time interval.

Basic camera studies to determine the parameters for radioisotope dosage, anatomical orientation and circulation times were completed in three baboons using 133 Xenon (133 Xe), 99m Technetium labeled albumin microaggregates (99m TcAA), 1-8μ in size, and 99m Technetium pertechnetate (99m Tc-pertechnetate), in a small, high specific activity bolus.

Clinical camera studies have been performed on 20 patients with intracranial lesions which were visualized by 99m Tc brain scanning and/or carotid angiography. Following angiography, 133 Xe, 99m TcAA and 99m Tc-pertechnetate were sequentially injected via an internal carotid catheter. In the brain tumor patients, 133 Xe and 99m Tc-pertechnetate rapidly accumulated in the tumor region (2 sec.) and remained there for greater than 90 and 25 seconds, respectively, whereas, they are more rapidly cleared from the adjacent hemisphere. 99m TcAA, which remains intravascular, demonstrates the relative rate of blood flow through the brain tumor. 133 Xe shows the greatest and most prolonged radiodensity within the tumors.

In the 5 head trauma cases, all three radioisotopes demonstrated relative decreased radiodensity within the surgically proven regions of intracerebral hematoma and cerebral edema compared to the radioactivity seen in the uninvolved brain tissue. These results contrast markedly with the definite increased radiodensity seen in the brain tumors.

Preliminary results would indicate that the camera studies distinguish between brain tumors and hematomas in cases not clearly diagnosed by angiography.

Cinemicroscopic Studies of the Microcirculation:

The microvasculature of the rabbits omentum was used to ascertain the effects of changed electrical environment on small blood vessels. Placement of microelectrodes on either side of a parallel arteriole-small vein system was performed. The vessel segments were then subjected to an electrical field perpendicular to the direction of blood flow. In vessels with slowed flow, lamination of the blood with RBC's segregated from the plasma was seen. The cells layered toward the vessel wall nearer the anode. A second finding was the rapid oscillatory motion of the blood in vessels between the electrodes. Further investigation of laminar and oscillatory flow and its relation to the electrical environment of other variables is planned in the near future.

Human Brain Tumor Culture Studies:

Quantitative measurement of radioactive uptake by human brain tumor cells growing in tissue culture, without a vascular supply, permits assessment of cellular affinity for specific radioisotopes.

Viable brain tumor explants are grown on plasma clots in Rose Culture Chambers containing nutrient media. Time of optimum cell growth (9 to 21 days) and quantity of tissue is determined by measurement under phase microscopy. Cells are then incubated for 18 hrs. in fresh media containing 200-350μc 99m Technetium (Tc) pertechnetate or 197 mercury. After incubation, media is removed from the chamber; cells and clot are washed 5 times with isotonic saline before transfer from the chamber to a dram-vial for assay of radioactivity in a deep well counter. Simultaneously 2 control chambers containing plasma clots are similarly processed for determination of residual radioactivity on the clots. Radioactive uptake by the cells is then determined by subtracting counts per min.(CPM) of control clot from CPM of the clot plus cells.

Neoplasms studied with 99m Tc-pertech. are: medulloblastoma, meningioma, glioblastoma, astrocytoma and ependymoma. Preliminary results indicate that all cells have a statistically significant radioactive uptake; low grade astrocytomas exhibit less uptake (33.5% of total count) than meningiomas or glioblastomas (86.2% and 92.5% respectively). It would appear that tumor cells may take up the radioisotope independently of an altered microcirculation.

15. EXPECTED RESULTS IN FY 1969

Cerebral Hemisphere Hemodynamics:

The clinical program for studying the cerebral microcirculation of patients with brain pathology should be established using the ten probe scanner and x-ray image amplifier typs scintillation camera. Correlation will be made with electroencephalography and angiography. Patients with symptmatic neurological disorders and no angiographic abnormality will be carefully assessed for impairment of regional cortical blood flow.

Human Brain Tumor Culture and Rabbit Implantation:

The human brain tumor cell cultures should indicate possible specific cell type affinity per se for certain radioisotopes. Factors influencing this affinity will be investigated.

Following the development of the in vitro cell tagging technic similar type cells will be implanted stereotactically in the rabbit brain for in vivo studies. This will add several more variables to the cell tagging mass and more closely simulate the clinical problem. The role of the blood-brain barrier in the radiolabeling of the tumor site will be investigated after suitable human tumor cell implants have been developed.

Cinemicrophotographic Studies:

Continuing studies are anticipated on the effect of microelectric currents on small arteries and arterioles of exteriorized rabbit omentum and on the pial vessels of the cerebral cortex. Changes following intracarotid injection of vasodilating drugs, angiographic agents and RAMA suspensions will be recorded.

16. EXPECTED RESULTS IN 1970

The studies outlined for FY 1968 will be continued in FY 1970. Various facets of each problem as they arise will be pursued to a reasonable understanding and solution.

Cinescintiangiography will be used routinely to supplement radiocontrast angiography in the evaluation of patients with cerebrovascular disease.

Yet unknown test agents with high affinity for specific types of *in vitro* human brain tumor cells will be tried in the tumor cell implant and animals. When proven successful *in vivo*, the test agents will be tried clinically whenever they are made available in suitable form.

Further observations using improved cinemicrophotographic technics will be made in animals and cell culture chambers and recorded on film.

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.:	4. Date Prepared:
06-10-00	April - 1968
5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA
7. Person in Charge:	8. Project Term:
Norman S. MacDonald	From: 1955 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	4 ½	4	4
(b) Other Tech.	1	1	1
Total	5 ½	5	5

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 67,800	$ 59,700	$ 62,100
(b) Materials & Services	7,700	7,700	7,700
(c) Indirect Expenses *	(5%) 49,600	(4%) 40,300	(4%) 43,200
Total	$ 125,100	$ 107,700	$ 113,000

* 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 1968

MacDonald, N.S., Hayes, M., and Figueroa, W.G. Medical Utility of a Total Body Counter - A Four Year Assessment. J. Nuc. Med. 8: 588-600,1967.

MacDonald, N.S., Hayes, M., and James, E. Radionuclide Contamination Encountered in Personnel Operating a Cyclotron with 50 Mev Protons, Symposium on Diagnosis and Treatment of Deposited Radionuclides, Battelle Memorial Institute, 1967 (In press).

MacDonald, N.S. Early Movements of Bone Seeking Radionuclides in the Dog's Leg After Intravenous Injection. Third Conference on Biology of Hard Tissue. New York Acad. of Sciences, 1967 (In press).

Hayes, M., Larson, W.M. Jr., and MacDonald, N.S. Measurement of Loss of Labeled Albumin in Children by Means of Total Body Counting. J. Nuc. Med. 8, 330, 1967.

12. SCOPE OF THE PROJECT

Radioactive nuclides have become extremely valuable as tracers of physiologic processes and as clinical diagnostic aids. The objectives of this project are: to ascertain the mechanisms by which various radionuclides are deposited in, and cleared from, body soft tissues and bone; to study the metabolism of radioactive pharmaceutical agents developed for diagnosis of human diseases; to study by radioactive nuclide labeling methods the behavior of various atomic species normally present in the human body (Ca,Na, K, CL, Mg, Fe,etc.,) seeking correlations of abnormal behavior with disease states; and to devise tests and techniques of aid in the diagnosis and treatment of such metabolic derangements.

A Total Body Counter Facility is maintained to detect and identify extremely small quantities of radioactive materials in living human beings. It is used to monitor the radioactive body burdens in children and adults resulting from contamination of the environment or occupational exposure. In addition, the Counter Facility is used in clinical research wherein tracer amounts of radioactive materials are intentionally administered. The amounts of radioactivity used in the 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 Cancer Research Hospital; Johns Hopkins School of Medicine; Brookhaven National Laboratory; University of Rochester AEP; Donner Laboratory, University of California; Los Alamos Scientific Laboratory; University of Utah AEP.

14. TECHNICAL PROGRESS IN FY 1968

Total body monitoring of radioactivity after intravenous administration of human serum albumin labeled with Iodine-131 was confirmed as a practical and easy method for estimating exudative and/or metabolic losses of albumin in humans. The sensitivity of the UCLA Total Body Counter (TBC) permits replicate measurements during a three week period

following injection of only 0.05-0.10 microcuries. This delivers less than 10 millirads of ionizing radiation and is an acceptable level for children. During the period of 2-10 days post-injection, the retention half time was 8-13 days in 9 healthy children. Of seven ill children, four showed half times of 2-6 days. The other 3 were undergoing intensive therapeutic treatments and had albumin retention half times in the normal range.

The Total Body Counter can also be used to determine the absorption and retention of dietary iron by monitoring the radioactivity in the patient following oral administration of ferrous citrate mixed with as little as 0.1 microcurie of Iron-59. In order to demonstrate the validity of the TBC method, 22 determinations of oral iron absorption were made on 14 hospital patients. In each case, total stools were also collected and the excreted activity measured. The values for iron absorption calculated by the stool assay method and by the TBC method were in good agreement. The TBC method is much simpler, far less tedious and equally accurate. (These studies were conducted in collaboration with Doctor W.G. Figueroa of the Department of Medicine).

One hundred and twenty individual measurements with the TBC on people living in Southern California showed that the mean body burden of radioactive cesium-137 continued to decrease from the peak in 1964-65 of around 95 picocuries per gram of body potassium to a level of around 28 p Ci/gK at the end of 1967. In monitoring several persons who operate the UCLA Physics Department Cyclotron, two instances of low-level contamination with Manganese-54 and Beryllium-7 were uncovered. These were attributed to inhalation of irradiation products produced by the 50 Mev proton beam of the machine.

15. EXPECTED RESULTS IN FY 1969

A considerable mass of data has been collected on the rates of appearance of various bone-seeking radionuclides in the soft tissues and bones of humans and dogs immediately following rapid intravenous injection. These kinetic data, called "osteograms", will be analysed by a digital computer program currently being written in order to estimate the rates of the various physiologic processes responsible for the net accumulation of activity in skeletal tissue during the first hour.

The Total Body Counter Facility will continue to be used to devise new methods for obtaining information of value in the medical diagnosis of human diseases. For example, a study of the rates of loss of gamma globulin (labeled with radioactive Iodine-131) from the body will be undertaken in normal and in hospitalized patients, in collaboration with Doctors Levy and Klinenberg of the Department of Medicine. The regular TBC monitoring of University students and employees who work with gamma-emitting nuclides will also continue.

As new diagnostic agents are developed by the Radiopharmaceutical Development Section they will be studied in animals in order to evaluate toxicity, distribution in body tissues, routes and rates of excretion, and other features of metabolic behavior.

16. EXPECTED RESULTS IN FY 1970

The same types of research activity will be continued - namely, studies of the metabolic behavior of various important substances normally present in the body, and of chemical agents administered to humans for diagnostic purposes. Thus, investigation of the oral absorption and retention of calcium and iron in humans will be continued. Multi-channel tape recording of radioisotope uptake measurements using multiple, short-lived nuclides and computer analysis of the data so obtained should elucidate the kinetics of some of the intercompartmental transfers which normal body electrolytes undergo. (Sodium, potassium, calcium, magnesium, iron and chloride, will be among the ions studied). Production of a number of short-lived radionuclides by the Biomedical Cyclotron should be underway by 1970. Consequently there will be an intensification of efforts devoted to metabolic studies of these materials and of chemical agents tagged with these nuclides.

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:
Radiodiagnostic Agent Development

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA and Harbor General Hospital

7. Person in Charge:
George V. Taplin, M.D. and
Norman S. MacDonald

8. Project Term:
From: 1967 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	1	2 ½	3
(b) Other Tech.	¼	-	-
Total	1 ¼	2 ½	3

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 22,100	$ 41,000	$ 45,500
(b) Materials & Services	6,200	5,500	8,900
(c) Indirect Expenses *	(2%) 19,900	(3%) 30,200	(3%) 32,400
Total	$ 48,200	$ 76,700	$ 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 1968

Taplin, G.V., Swanson, L.A., and Hayes, M. Recent Advances in Kidney Scanning. Lahey Clinic Foundation Bulletin, 16:361-372, 1967.

Hayes, M., and MacDonald, N.S. Evaluation of Short-lived Radiopharmaceutical Agents for Sequential and Conventional Kidney Scanning. J. Nuc. Med. May, 1968 (abstract)

12. SCOPE OF THE PROJECT

The primary function of this project is to develop and supply new radioactive diagnostic agents of direct value to both the basic and the clinical research activities in Nuclear Medicine. A secondary function is to evaluate existing agents, including those which are commercially available, with the purpose of improving their usefulness. These functions entail: planning and execution of appropriate chemical reactions; confirmation of the identity and purity of the desired product; assay of the radioactivity of the product; preparation of the product in sterile, pyrogen-free form suitable for parenteral administration to humans; and the design and assembly of suitable equipment so that these operations (often at the level of 100-200 millicuries) can be carried out with minimal radiation exposure to personnel.

13. RELATIONSHIP TO OTHER PROJECTS

Similar work is being performed at Argonne Cancer Research Hospital (Harper); Johns Hopkins Medical Center (Stern, Wagner); Veterans Administration Hospital, Los Angeles (Tubis); Brookhaven National Laboratory Medical Department (Richards); Nuclear Medicine Institute Sao Paulo, Brazil (Tede, Eston); University of Heidelberg, (Scheer).

14. TECHNICAL PROGRESS IN FY 1968

Modifications were made in the procedure for labeling human serum albumin with technetium - 99m, both in chemical conditions and in the equipment used. Tagging is routinely performed daily and the yields are consistently 85-90%. Adjustments of pH, transfers of the solution, treatment with ion exchange resin and sterilization by filbration are now done by a "semi-remote handling" technique in which the operators hands and body are well shielded. New equipment for aggregating labeled albumin by combined ultrasound agitation, heating and mechanical shaking was assembled and put in operation. Quality control methods were devised to insure the sterility and freedom from pyrogens of all preparations destined for use in humans and laboratory animals.

Five chemical complexes of short-lived radionuclides were prepared and evaluated as pharmaceutical agents for kidney scanning. These were: 99m technetium-Fe-ethylene dramine tetraacetate; 99m Tc-Fe-diethylene triamine pentaacetate; 113m Indium-EDTA; 99m Tc-Fe-glucose; and 99m Tc-Fe-citrate complexes. Radioisotope renograms, urinary clearances and rapid sequential scans all indicated that these agents are rapidly cleared from the blood and excreted into the urine at rates which are very close to labeled iothalamate (an inulin substitute known to be

cleared by glomerular filtration). For example, urinary output was 45-55% of the injected dose within the first hour, whereas uncomplexed pertechnetate ion clearance was less than 2% in that time. These agents show great promise for kidney scanning, both by rapid sequential and by conventional methods, and perhaps in studying the rapid circulatory dynamics of major blood pools such as the brain and heart. Their rapid excretion and short physical half-life greatly reduce radiation exposures to the patient.

Comparisons were made in dogs of the rates of clearance from the lungs of intravenously administered particles of human serum albumin labeled with technetium 99m and particles of a similar size distribution but labeled with iodine-131. Under the microscope the iodine labeled aggregates appear more dense and tougher. Preliminary results suggest that the Tc-labeled particles clear from lung parenchyma more rapidly than Iodine labeled particles of a similar size, probably because the former are more fragile.

15. EXPECTED RESULTS IN FY 1969

Efforts to improve the control of particle size during preparation of aggregates of human serum albumin labeled with various radionuclides will be continued. The potentialities of microwave heating as a method of preparing macro - and micro - aggregates which have been explored briefly will be developed further. Improvements will be made in the techniques of preparing radiopharmaceutical agents from the aspects of personnel radiation protection and quality control (sterility, non-pyrogenicity, purity, etc) so that all regulations regarding radiopharmaceuticals applied by the Federal Drug Administration can be complied with.

Continued efforts will be directed toward labeling various chemical substances with short-lived radionuclides for evaluation as clinical diagnostic agents by other investigators in this Laboratory and at the UCLA Medical Center and affiliated institutions. For example, efforts will be made to prepare radioactively labeled substances which are rapidly cleared from the blood by renal tubular secretion (in contrast to iothalamate and the EDTA chelates which are removed by glomerular filtration). Availability of such agents would make possible the differential diagnosis of several renal disorders. Similar work will be aimed at developing improved radioactive agents for scanning the pancreas.

16. EXPECTED RESULTS IN FY 1970

The activities of this section will continue along the same lines as in the previous years. However, if the Biomedical Cyclotron Facility is established there will be an intensification of emphasis on techniques for preparing agents labeled with nuclides of short half-life because of the anticipated production of such isotopes with this machine. Many new problems will arise in the process of converting the desired activity in the irradiated target to the labeled radiopharmaceutical ready for injection into a human patient. However, expert consultative advice and technical assistance will be available in the Laboratory to help solve these problems.

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 Studies - Short Lived Isotopes

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Milo Webber, M.D.

8. Project Term:
From: 1967 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2	2 ½	3 ½
(b) Other Tech.	-	-	-
Total	2	2 ½	3 ½

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 21,400	$ 31,100	$ 44,500
(b) Materials & Services	4,900	7,900	9,100
(c) Indirect Expenses *	(2%) 19,800	(2%) 20,100	(3%) 32,400
Total	$ 46,100	$ 59,100	$ 86,000

* 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 1968

Schwabe, Arthur D., Valdivieso, Vicente D., Merrill, Samuel, Ortega, Carlos, Bennett, Leslie R., and Thompson, James C., "Studies on the Intestinal Absorption of a Medium Chain Fat (Trioctanoin) in Normal and Pancreatectomized Dogs", The American Journal of Digestive Diseases 2:11:1114-1121, November 1967.

Webber, Milo M., Moderator; Bloomer, William E., Crandall, Paul H., Drinkard, James, Glober, Gary, Herbst, Harry H., Johnson, DeLores E., Killeffer, Fred A., McAlpin, Rex N., Pops, Martin A., Stern, W. Eugene, Swanson, Leonard, Taplin, George V., and Weidner, William A., "The UCLA Interdepartmental Conference: The Use of Radioisotope Scanning in Medical Diagnosis; Applications in Diseases of Brain, Lung, Liver, and Heart", Annals of Internal Medicine 67:5:1059-1083, November 1967.

Webber, Milo M., and Bennett, L. R., "Full Size Readout on the Gamma Camera", pp. 105-108, Progress in Biomedical Engineering, eds. Lawrence J. Fogel and Frederick W. George, London: Macmillian and Company, Ltd., 1967.

Webber, Milo M., "Footnotes on Radioisotopes", in Schinz, H. R., Roentgen Diagnosis, Vol I., second American edition, arranged and edited by Leo G. Rigler, New York: Grune and Stratton, 1967.

Webber, Milo M. and Bennett, L. R., "Full-Size Scintiphotos from the Scintillation Camera", J. Nuc. Med. 8:5:2:403, May 1967. (abstract)

Webber, M. M., Bennett, L. R., Wagner, Joseph, and Patton, Dennis L., "Demonstration of the Vascularity of the Femoral Head", J. Nuc. Med. 8:5:2:403-404, May 1967. (abstract)

Webber, Milo M., Dewig, Elwood, and Smith, Raymond L., "Electronic Analogue Contrast Enhancement", J. Nuc. Med. 8:5:2:404, May 1967. (abstract)

Surprenant, E. L., Bennett, L. R., Webber, M. M., and Wilson, A. F., "Measurement of Regional Pulmonary Ventilation with Radioxenon and the Anger Camera", J. Nuc. Med. 8:5:2:400, May 1967. (abstract)

Surprenant, E. L., Browning, N., Bennett, L. R., and Webber, M. M., "The Significance of Shine-through on the Side View Lung Scan", J. Nuc. Med. 8:5:2:400, May 1967. (abstract)

Awad, William, Boake, Rex C., Bennett, Leslie R., and Martin, Donald C., "Double Isotope Scan in Kidney Transplantation", American Journal of Surgery (in press).

Surprenant, Edgar L., Bennett, Leslie R., and Webber, Milo M., "Technetium Lung Scanning", Int. J. Applied Radiation and Biology (in press).

12. SCOPE OF THE PROJECT

This project was established in FY 1968 in anticipation of the enlargement of the Nuclear Medicine space, facilities, and research activities in the Medical Center in the Department of Radiology, to develop a well balanced Nuclear Medicine research program coordinating the activities of the Nuclear Medicine Division in the Laboratory with those at Harbor Hospital and the Medical Center.

Initially, the research activities of the Section are devoted to clinical applications of Tc-99m compounds in organ scanning using the Anger camera currently available in the Medical Center. Heart and blood pool scanning are performed with technetium labeled human serum albumin in millicurie doses, permitting far better visualization of the heart chambers and great vessels than possible with radionuclides. The same agent is used for placental scanning in the diagnosis of placenta previa.

Colloidal aggregates of technetium labeled serum albumin and/or technetium-99m sulphur colloids are being investigated for liver, spleen and bone marrow scanning. Again, the technetium-99m labeled compounds have great potential advantages over colloidal radiogold and colloidal albumin aggregates labeled with I-131. They can be given in doses up to 20 times greater than the former compounds while giving less radiation exposure to the patient. The accompanying increase in photon emission can greatly improve organ vizualization with both conventional and camera scanning techniques. For bone marrow studies it makes scanning a practical procedure because with colloidal gold the dose to the patient is far above acceptable exposure levels.

Another area of clinical research is in bone scanning with Fluorine-18 and Strontium-87m labeled compounds. These short-lived nuclides have great potential advantage over currently used Strontium-85 and Calcium-47. Studies with the latter have indicated much promise for bone scanning in the early detection of primary and metastatic bone tumors and for the study of the healing of fractures. Scanning methods can detect bone lesions long before they are recognizable radiographically. Again the short-lived Fluorine and Strontium-87m nuclides have great potential advantage in that they can be used in much larger doses and thereby improve the sensitivity of scanning methods.

Another study initiated in this Section is the investigation of specific antibody labeling as a possible tool in organ scanning techniques. Anti-fibrin antibodies have been investigated by Dr. William Bale and associates for the treatment of tumors. Although the tumor specificity has not been developed sufficiently for this purpose, it is entirely adequate for the detection of tumors in various parts of the body by radioisotope scanning. Another application of the antifibrin antibodies is in the study of venous thrombosis and subsequent pulmonary embolism. It is possible that there may be sufficient selective localization of the antibodies at sites of thrombus formation in the legs and pelvis to identify such sites by scanning in patients with pre-existing diseases which make them far more susceptible to pulmonary embolism. Another advantage of detecting the site of thrombus formation is in the surgical prevention of embolism by venous ligation. In

addition to exploiting the uses of antifibrin antibodies, the preparation of specific organ antibody for scanning is an area of promise that remains to be explored.

13. RELATIONSHIP TO OTHER PROJECTS

The project is directly related to the activities of the new Section on Radiodiagnostic Agent Development and to those of the Basic Nuclear Medicine Section. Similar studies are being conducted in bone marrow scanning with colloidal radionuclides at Oak Ridge Institute of Nuclear Studies; short-lived technetium compounds are being produced and investigated at the Brookhaven National Laboratory, Upton, N. Y.; the antifibrin antibody studies were initially developed under Dr. William Bale at the University of Rochester, N. Y.; related fibrinogen compounds are being produced and studied at the University of Pisa, Italy under the direction of Dr. Luigi Donato; basic studies and developments of various technetium-99m labeled compounds are under investigation both in basic studies and for clinical applications at the Argonne Cancer Institute in Chicago under the direction of Dr. Paul W. Harper; bone scanning with Fluorine-18 compounds was developed first at Roswell Park Memorial Hospital by Drs. Mone Blau and Merrill Bender; recent developments in Fluorine compounds for bone scanning are under way at the University of Michigan Department of Nuclear Medicine under the direction of Dr. William Beierwaltes and his associates.

14. TECHNICAL PROGRESS IN FY 1968

Attention this year was devoted initially to the development and refinement of technetium-labeling of albumin. The parameters of this preparation procedure have been carefully analyzed, and steps have been taken to insure quality and reproducibility of the technique. Albumin prepared in this way has been used to delineate the cardiac blood pool with a remarkable degree of success. The cardiac blood pool scan has proven to be a valuable clinical procedure and is now routinely utilized in cases where a question of pericardial effusion, aneurysm, or vascular anomaly exists.

Ventilation scanning technique utilizing Xenon-133 has been developed and shows much promise as a useful clinical tool. The ventilation pattern, as distinct from the pulmonary perfusion pattern, is now available. The studies have been performed using the scintillation camera because the picture or "scan" must be made while the patient holds his breath. Correlation between perfusion and ventilation scans has been performed on patients suffering from asthma, emphysema, and other pulmonary diseases.

The technique of ventilation scanning using Xenon-133 is also in the process of evaluation as a tool for evaluating the function of the transplanted lung in dogs.

A comparison is under way between non-radioactive Xenon as a contrast agent in chest radiography and the radioxenon technique. By photographic subtraction of chest films with air and then with Xenon, the ventilation pattern may be appreciated.

The particle size distribution of technetium sulfide is currently under study. Ultracentrifugation and electron microscope methods are being investigated.

Macro and micro aggregates of albumin for demonstration of intravascular endothelial lesions are being studied. It is known that intravascular trauma, as caused by catheters and cautery will result in tracer accumulation. The extension of this technique to demonstrate thrombophlebitis and intra-arterial atherosclerotic ulcers is contemplated.

Studies have been continued on the use of Fluorine-18 as a bone scanning agent. Results to date indicate that fluorine will probably become the isotope of choice in scanning for bone lesions.

15. EXPECTED RESULTS IN FY 1969

The evaluation of small particle size radiotechnetium sulfide and micro-aggregates of albumin as well as stannous hydroxide or similar colloidal substances will be investigated as the agent for demonstration of the bone marrow phagocyte distribution within the body. The success of this study depends upon the particle size distribution and reproducibility of size from batch to batch. Ultracentrifugation and electron microscope methods may be used to verify uniformity. It may be possible to utilize these micro-particles to demonstrate the viability of the femoral head following fracture of the femoral neck. This will be of great value in the management of such fractures.

The usefulness of intravascular injection of isotope tracers to demonstrate areas of tumor will be pursued. Japanese investigators report success in promoting uptake of tracer by intra-arterial injection of macro-aggregates of radioiodinated albumin. This technique will be evaluated using radiotechnetium labeled albumin and other short-lived tracers.

Xenon-133 studies in various clinical conditions will be continued.

Studies on the use of Fluorine-18 tagged compounds as scanning agents will be continued.

16. EXPECTED RESULTS IN FY 1970

The usefulness of short-lived gas phase tracers will be evaluated in conjunction with the projected Cyclotron Facility. These are expected to be useful in the evaluation of pulmonary function. Compounds of Fluorine-18 and other short-lived positron emitters will be studied in relation to clinical usefulness.

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:
Biomedical Cyclotron Facility

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

7. Person in Charge:
Benedict Cassen and
Norman S. MacDonald

8. Project Term:
From: 1969 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	0	½	1 ¾
(b) Other Tech.	0	-	-
Total	0	½	1 ¾

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ -0-	$ 6,900	$ 21,300
(b) Materials & Services	-0-	-0-	9,200
(c) Indirect Expenses *	-0-	(1%) 10,100	(1%) 10,800
Total	$ -0-	$ 17,000	$ 41,300

* 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 1968

None

12. SCOPE OF THE PROJECT

The mission of this new project is to install and operate a cyclotron facility to provide radionuclides and beams of low energy positively charged particles, and neutrons for biomedical research. The main emphasis will be on the production of short-lived radionuclides for physiologic studies and clinical research in Nuclear Medicine. These materials will be made available to medical research investigators, not only at UCLA but also at several other hospitals in the community. Other activities will include neutron activation analyses of materials for other research programs in the Laboratory and (as time permits) in other departments. Charged particle beams (protons, deuterons,helium-3 ions) will be available for Laboratory investigations in radiobiology and radiation chemistry.

13. RELATIONSHIP TO OTHER PROJECTS

In addition to the part-time biomedical usage of the cyclotrons at Lawrence Radiation Laboratory and Donner Laboratory (University of California - Berkeley), machines designed for biomedical applications are in operation at Washington University (Ter Pogossian); Sloan-Kettering Medical Institute (Laughlin). Argonne Cancer Research Hospital (Harper) will probably install a cyclotron during 1969.

14. TECHNICAL PROGRESS IN FY 1968

The technical proposal and request for funds were drawn up and submitted. Preliminary inspection indicated that a site on the Laboratory grounds is feasible and could take advantage of certain concrete shielding walls of the existing Cobalt-60 Irradiation Facility. An alternate site at the Center for Health Sciences was identified, but non-AEC funds would be required for construction at this site. A search for possible sources of such funds was initiated with the help of the Office of the Dean of the Medical School and the Department of Radiology. Two visits to the Cyclotron Corporation (Berkeley, California) were made for the purpose of examining the equipment and performing trial beam irradiations of several target materials.

15. EXPECTED RESULTS IN FY 1969

If the proposal is approved and funds are allocated near the beginning of FY 1969, it is entirely possible that preparation of the site, construction, and delivery of the cyclotron can be accomplished in the succeeding 10-12 months.

16. EXPECTED RESULTS IN FY 1970

It is anticipated that "shake-down" runs, training of operators and preparation of several different target assemblies will be completed during the first quarter of FY 1970. Iodine-123 and Iron-52 will probably be the first nuclides scheduled for production. By the latter half of FY 1970 the facility should be in full operation.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Mammalian Radiobiology

3. AEC Budget Activity No.:
06-10-00

4. Date Prepared:
April - 1968

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

6. Working Location:
UCLA

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

8. Project Term:
From: 1954 To: Continuing

9. Man Years	FY 1968	FY 1969	FY 1970
(a) Scientific	2 1/2	2 1/2	2 1/2
(b) Other Tech.	1/4	-	-
Total	2 3/4	2 1/2	2 1/2

10. Costs	FY 1968	FY 1969	FY 1970
(a) Direct Salaries	$ 30,200	$ 30,800	$ 31,900
(b) Materials & Services	3,000	4,000	4,500
(c) Indirect Expenses * (2%)	19,900 (2%)	20,100 (2%)	21,600
Total	$ 53,100	$ 54,900	$ 58,000

* 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 1968

Hennessy, Thomas, G., Stern, W. Eugene, and Herrick, Stephen E.: Cerebellar Hemangioblastoma: Erythropoietic Activity by Radioiron Assay, J. Nuc. Med. 8, 601-606, 1967.

12. SCOPE OF THE PROJECT

The broad area towards which the research is directed is elucidating the control mechanisms of red cell production in mammals including man. Since iron is a major component of the red cell and since radioactive iron became available for a label, much of the earlier work was aimed at methods of applying this label in the study of the erythropoietic system. Noteworthy in much earlier work was the development with R. L. Huff of Ferrokinetics and the red cell uptake technique for the study of radiation damage to the bone marrow.

The particular facets of the broad problem stated above that are being studied are the hormonal aspects of erythropoietic control, in particular erythropoietin levels and their relation to disease states, including radiation damage to the bone marrow. A more complete understanding of erythropoietin should help to further explain aplastic anemia of which radiation injury of the blood forming organ is one class.

13. RELATIONSHIP TO OTHER PROJECTS

Studies on Fe^{59} and erythropoietin are currently being conducted at Lawrence Radiation Laboratory, University of California, Berkeley, Argonne Cancer Hospital, Chicago, NIH Hematology Section, Bethesda, Maryland as well as many other institutions.

14. TECHNICAL PROGRESS IN FY 1968

During this year the most significant development is the demonstration of a possible inhibitor present in mouse plasma for the erythropoietin present in aplastic anemia serum. This demonstration has been accomplished in short term bone marrow cultures but not in standard polycythemic mouse *in vivo* assays. It could represent an antibody reaction with the antibody present in the aplastic serum but which becomes activated by normal serum. Further work is required to elucidate the exact mechanism of the observed phenomena.

In addition during the year we have changed from using a hypertransfused mouse for our erythropoietin assays to a polycythemic mouse produced by a specially fabricated silicon rubber membrane altitude cage. This permits a faster and less costly production of assay mice.

A pilot study in doing Ferrokinetic studies with stable iron isotope has been terminated. In the pilot study it was shown that such studies would be feasible but excessively costly due to the amount of nuclear reactor time required.

Two additional studies were done during this year adding to our studies on cerebellar hemangioblastoma which were published this year in the Journal of Nuclear Medicine.

15. EXPECTED RESULTS IN FY 1969

Investigation this year will center primarily on the elucidation of the mechanism of action of erythropoietin inhibitor. Studies on erythropoietin producing tumor will continue as such human cases become available.

16. EXPECTED RESULTS IN FY 1970

Studies will continue along the lines discussed above without any major change in scope or level of the program.