MASTER

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

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

RESEARCH AND DEVELOPMENT PROGRAM
FISCAL YEAR 1972

APRIL-1970

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LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY
UNIVERSITY OF CALIFORNIA, LOS ANGELES
CONTRACT AT(04-1) GEN-12

U. S. Atomic Energy Commission
SAN FRANCISCO OPERATIONS OFFICE

Biology and Medicine
PROGRAM

RESEARCH AND DEVELOPMENT PROGRAM

INTRODUCTORY STATEMENT

GENERAL

The main Laboratory objectives are to adequately support our strong research programs rather than expand them, given the present budget stringencies. Nevertheless, several important shifts in our programs are expected to occur during the next two years within the limits of available funds. The most significant of these in their order of priority are:

1. Strengthening of our Environmental Radiation Research by recruitment of two or three new staff and expected closer interaction with campus ecological programs.

2. Initiation of research programs related to the new Biomedical Cyclotron Facility and expansion of complementary work with radiopharmaceuticals.

3. Further expansion in the area of Developmental Biology as budgets permit. Research electronmicroscopy may be the most pressing need.

4. Initiation of a new research program replacing that of Dr. Cassen who will reach mandatory retirement age by November 1972.

Additional discussion of these changes is found below.

An important new aspect for Laboratory activities is the increasing integration of our staff into the Campus academic affairs and the utilization of Campus resources in pursuing our research objectives. Contributory to this end is interaction and advice of the Laboratory Advisory Committee appointed by the Chancellor to advise the Director on Laboratory matters; the strong support of the Chancellor's office for Laboratory programs; and the implementation of the Laboratory's Special FTE's providing full professorial series privileges (except that tenure is related to the Contract) when implemented through an academic department.

There are many subtle advantages which will accrue to the Laboratory through these closer academic ties. To mention one, the involvement of graduate students and post-doctoral fellows in our programs will be a distinct advantage.

Chancellor's office support includes salary contributions to the Laboratory, partial funding for the cyclotron building, strengthening our recruitment posture by guaranteeing support of positions and subtle pressure, as needed, to develop more favorable atmosphere for development of Laboratory and Departmental mutual research interests.

Regarding the Special FTE's, it is present Laboratory strategy to utilize about half of the ten positions for existing staff, where possible, and apply the remainder for new recruitment. The following Special FTE's have been implemented:

For Current Staff

	Lab Division	Department
1. Dr. Cassen	Biophysics	Biophysics
2. Dr. MacDonald	Nuclear Medicine	Radiology
3. Dr. Taplin	Nuclear Medicine	Radiology
4. Dr. Fulco	Biochemistry	Biological Chemistry
5. Dr. de Vellis	Radiobiology	Anatomy

For Recruitment

1. Dr. Herschman	Biochemistry	Biological Chemistry

DISCUSSION OF PROGRAMS

1. Ecology

The recruitment of 2 or 3 new staff is planned for the ecology program to provide greater strength in key areas. It is planned to use some of the special FTE's for this recruitment in cooperation with appropriate departments. Pending a successful staff addition on our part, the Chancellor plans to make a regular FTE available to the Department of Botany for the recruitment of a man whose research interests would contribute to our program. Needed areas of competence in our program are visualized as modeling and vertebrate ecology. We expect to be working closely with Dr. Jerry Davis of the Nevada Operations Office in meeting applied radioecology objectives at the Nevada Test Site. We will also be participating in aspects of the Desert Biome studies under the International Biological Program. At the Laboratory administrative level, consideration is being given to developing complementary ecological studies on power plant siting problems. Activity in this area would be dependent on our assessment of research needs, the feasibility of some commitment to these problems by our staff, and probably support from industry or state agencies.

Assessment of the progress and relevance of our existing ecological programs will continue. The radiation ecology studies in Rock Valley are yielding results which are of great potential scientific interest which we hope to be able to exploit fully. Observations being made on the effects of long-term, relatively low-level radiation provide an interface for interaction between staff in radiation biology and ecology.

Improvement of our ecological program is the number one priority of the Laboratory, partly because of the significance of these problems in national planning of

various types and its importance as an AEC mission priority. The time now seems to be propitious to have the Laboratory contribute effectively to a much more vigorous campus program in ecology. The Director is a member of an interdisciplinary committee involving students as well as faculty to advise the Chancellor on environmental research goals.

2. Nuclear Medicine

This program has been outstandingly successful and capabilities are being further enhanced through the cyclotron program starting in the Fall of 1970. Drs. Cassen and MacDonald co-chair a committee responsible for its safe and effective operation. Complementing this activity and others in Nuclear Medicine will be expanded activities in radiopharmaceuticals. Support for additional work in this area is expected from industry. The transfer of research programs from Warren Hall (the Laboratory building) to the Medical Center is expected to facilitate closer interaction with related programs conducted by the Department of Radiology and be advantageous from the point of view of proximity to clinical resources.

It is expected that the program on cerebral hemodynamics, conducted by Dr. Kennady, will be completed by the end of FY 71 or 72 and be replaced by a program in one of several presently attractive areas under consideration.

Under University regulations, Dr. Cassen, who has complemented work on Nuclear Medicine through instrumentation research, would normally retire July 1, 1970. However, because his mental acuity remains undiminished and his health is apparently good, he desires to remain active, and particularly because his training and background are especially appropriate to the initiation of our cyclotron research project, authorization has been obtained from the University administration to extend his employment on a year-to-year basis. We expect him to remain on salary, if he remains in good health and vigor, until November 1, 1972. Dr. Cassen will be devoting a large amount of his time to planning and developing initial projects with the cyclotron. During this period we also expect him to be drawing his current research projects to conclusion although he may well remain active in research after November 1, 1972. Among current research activities, work under budget category 06-02-08 will be largely confined to refinements in the sectional scanner and cell characterization through holography while work under budget category 06-01-01 will continue actively on biophysical cellular studies. Because of Laboratory budget problems, and after consultation with various DBM staff, it was agreed that in order to sustain other strong programs (and because Dr. Cassen should, in fact, be drawing his programs together) expediency requires a reduction in the level of support for Dr. Cassen and reprogramming of these funds to support programs in budget category 06-01-02 during FY 1971 and FY 1972.

It has been our plan (again in consultation with DBM staff) to commit Dr. Cassen's research resources to a new program to begin possibly in FY 1972 or FY 1973. The program may be in an area of instrumentation supportive of nuclear medicine or radiation biology. We visualize that the candidate should be well trained in the physical sciences and radiation theory. Dr. Cassen presently chairs a committee to study the Laboratory needs in these areas and seek out candidates. The present relocation of part of the present support of Dr. Cassen reduces our flexibility to proceed along the lines just described. However, it is our

intention to have Dr. Cassen's committee proceed with its assignment. We trust that the necessary funding to support the new program can be generated when we have our candidate.

A tabulation of practical clinical applications resulting from research in this Laboratory is most impressive. Continued progress on a number of complex and important medical problems is expected. Furthermore, work from this area will be given proper attention in our publicity releases because of public appreciation.

3. Radiation Biology

This is a program we feel needs strengthening when budgets permit. The research objectives are of very fundamental nature and research objectives in this area contribute to other programs and vice versa. Dr. Howton, from the Biochemistry Division, also conducts work in radiation chemistry. It is expected that Dr. de Vellis will transfer from this division to Developmental Biology. Dr. Byfield, whom we expect to add to the Division of Radiation Biology, has interests in developmental problems. Thus, we expect considerable interaction between this division and Developmental Biology. At the present time, Drs. Myers and Ward are studying radiation effects on relatively simple molecules although the reactions are very complex. Dr. Strickland is doing sophisticated biophysical work on the relation between structure and function of more complex molecules. Structural effects induced by radiation-initiated reactions now appear to be amenable to investigation. The addition of Dr. Byfield will extend radiation biology research to the cellular and organismal level while an understanding of the radiation effects will continue to be sought at the physiological and molecular level. Thus, the program in this level, although small, will be concerned with all the levels of biological organization.

4. Biochemistry - Developmental Biology

Most future planning of the Biochemistry Division presently revolves around the formation of a Developmental Biology group, eventually to become a separate division. This group will include Drs. Harary, Herschman, Gerschenson and deVellis, plus some postdoctoral fellows or visiting scientists. It is felt that while this group is already active and productive, it will eventually need at least one additional scientist as part of the permanent staff. In addition, it has become increasingly obvious that a research electron microscopist is a necessity, not only for the Developmental Biology research, but also for that of many other groups in the laboratory.

The impact of this group will not be limited to its own research but is being felt throughout the laboratory. In particular, the increasing involvement of most of the scientists in this area with cell culture techniques is a reflection of the interests and abilities of this group. This versatile and powerful technique is being used to solve such difficult problems as the nature of the malignant transformation, the effect of hormones on the liver and uterus, the formation of myelin and its relationship to diseases of the brain and the nature and properties of membranes. Most of these problems continue to remain with us because of their complexity, which defies simple means of solution. The cell culture technique seems to provide a profitable avenue of approach to them.

The relationships of the Biochemistry and Radiation Biology groups will continue to be productive. Dr. deVellis will be a member of the Developmental Biology

group while Dr. Howton will aid in Radiation Chemistry studies. Drs. Mead and Dhopeschwarkar will continue to cooperate with Drs. Cassen and Myers in studies on the effect of low-level radiation on permeability of the blood-brain barrier. The basic studies of Drs. Fulco and Nevenzel will aid in elucidating the effects of thermal pollution.

There is, in short, every reason to hope that, despite almost crippling budgetary problems, this will be another productive year.

COST INFORMATION:

The composition of costs and staffing for FY 1970, FY 1971, and FY 1972, are summarized below for the entire Biology and Medicine Program by major categories of expense.

	FY 1970	FY 1971	FY 1972
I. Costs: (Shown in Thousands)			
Salaries and Burden (Direct)	$1,399.8	$1,345.2	$1,427.8
Supplies, Travel, & General Expense	232.2	230.8	250.2
Indirect Costs	848.0	830.0	858.0
Total Operating Costs	$2,480.0	$2,406.0	$2,536.0
II. Manpower: (Shown as Full Time Equivalence)			
Direct Man Years	104	99	102
Direct Scientific Man Years	92	87	90
III. Cost Per Man Year Data: (Shown in Thousands)			
Cost Per Direct Man Year	$ 23.8	$ 24.3	$ 24.8
Cost Per Direct Scientific Man Year	$ 26.9	$ 27.6	$ 28.2

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

As will be noted in the individual project statements the method used at this Laboratory for allocating indirect costs to research projects consists of prorating total indirect costs on the basis of the percentage of total direct salary expense that each research group incurs. This method of assigning indirect costs is believed to be sufficiently accurate and appropriate for an organization of our size and relative uniformity of composition.

However, under this method of proration indirect costs are not specifically identifiable under individual research projects, and for this reason, the composition of indirect expense for the total Program is summarized below.

	FY 1970	FY 1971	FY 1972
Composition of Indirect Expense:			
I. Manpower (Shown as Full Time Equivalence):			
Administrative Services	30 ½	28 ½	28 ½
Technical Services	9 ½	7 ½	7 ½
Building Maintenance	7	6	6
Total Indirect Personnel	47	42	42
II. Costs (Shown in Thousands)			
Administrative Services	$ 345.2	$ 358.4	$ 373.7
Technical Services	122.5	94.8	99.4
Building Maintenance	68.3	62.8	63.9
Building Amortization	126.7	126.7	126.7
Utilities	59.0	63.0	67.0
U. C. Management Allowance	75.0	75.0	75.0
U. C. Accounting and Purchasing Services	34.0	35.0	37.0
Miscellaneous (Laundry, Postage, General Supplies, etc.)	17.3	14.3	15.3
Total Indirect Costs	$ 848.0	$ 830.0	$ 858.0

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 1970 COSTS	FY 1970 MAN YRS	FY 1971 COSTS	FY 1971 MAN YRS	FY 1972 COSTS	FY 1972 MAN YRS
06 01 01	Effects of Radiation on Living Organisms						
	Medical Physics Problems (Cassen)	$ 52.6	3 1/4	$ 28.8	2	$ 29.5	1 1/2
	Late Effects Radiobiology (Bennett)	45.4	1 3/4	30.2	1 1/2	30.5	1 1/2
	ACTIVITY TOTAL	$ 98.0	5	$ 59.0	3 1/2	$ 60.0	3
06 01 02	Molecular and Cellular Radiobiology						
	Enzyme Chemistry (Harary)	$ 125.8	4	$ 114.3	3 1/2	$ 115.4	3 1/2
	Macromolecular Chemistry (Simmons)	54.5	3	15.2	1/2	53.7	3
	General Metabolism (Mead)	68.1	4	72.0	4	73.9	4
	Organic Chemistry (Howton)	71.3	2 1/2	73.0	2 1/2	77.1	2 1/2
	Tracer Synthesis (Nevenzel)	29.3	1 1/2	45.4	2	46.0	2
	Chemical Radiobiology (Myers)	114.2	5	118.9	5	123.3	5
	Sub-Cellular Radiobiology (Ward)	54.1	2 1/2	55.9	2 1/2	58.6	2 1/2
	Cellular Radiobiology (Byfield)	0	0	.5	0	3.0	0
	Metabolic Radiobiology (Schjeide)	29.3	3/4	0	0	0	0
	Biosynthetic Control (Fulco)	39.3	1 1/2	52.8	2 1/2	63.1	2 1/2
	Physical Radiobiology (Strickland)	73.0	2 3/4	83.6	3	85.8	3
	Developmental Radiobiology (de Vellis)	57.8	3	72.7	3	75.7	2 3/4
	Developmental Biology (Herschman)	54.3	2 1/2	59.7	2 1/2	61.4	2 1/4
	ACTIVITY TOTAL	$ 771.0	33	$ 764.0	31	$ 837.0	33
06 02 04	Land and Fresh Water Environmental Sciences						
	Soil Factors (Nishita)	$ 96.6	4 1/4	$ 88.6	3 1/4	$ 89.7	3 1/4
	Plant Factors (Romney)	111.5	4 1/4	90.4	3 1/4	92.6	3 1/4
	Environmental Factors (Hawthorne)	82.8	3 1/4	68.4	2 1/4	54.1	2 1/4
	Plant Physiological Ecology (Wallace)	115.4	2 3/4	73.3	2 1/2	81.8	2 3/4
	Radiation Ecology (Maza-Acting)	78.4	2 3/4	70.6	2 3/4	80.2	3 1/4

	Ecology of the Nevada Test Site (Beatley)	69.9	3 1/4		72.3	3 1/4	45.6	1 3/4
	Radioecology (Turner)	94.5	4		70.9	3	89.1	3 1/4
	Chemical Problems (Wood)	138.9	6 1/4		113.7	5 1/4	107.6	5 1/4
	Physiology of Mineral Accumulation in Plants (Lunt)	26.2	1 1/4		28.3	1 1/4	29.1	1 1/4
	Quantitative Plant Ecology (Wallace-Acting)	31.0	1 1/4		80.9	3 1/4	92.9	4 1/4
	Physiological Ecology (Dainty)	28.8	1 1/4		19.5	3/4	19.8	3/4
	Soil Survey and Characterization (Hale-Acting)	0	0		29.5	1 1/4	31.1	1 1/4
	Distribution and Interrelationships of Elements in Biological Systems (Alexander)	0	0		23.6	1	24.4	1
	ACTIVITY TOTAL	$ 874.0	34 1/2	$	830.0	33 1/2	$ 838.0	33 1/2
06 02 08	Radiological & Health Physics & Instrumentation							
	Medical Physics Instrumentation (Cassen)	52.0	2 1/2		45.0	1 1/2	46.0	1 1/2
	ACTIVITY TOTAL	$ 52.0	2 1/2	$	45.0	1 1/2	$ 46.0	1 1/2
06 03 01	Cancer and Other Clinical Research							
	Clinical Nuclear Medicine (Taplin)	$ 126.1	5	$	143.0	6	$ 146.5	6
	Basic Nuclear Medicine (Poe)	56.4	3 1/4		70.2	3 1/4	74.3	3 1/4
	Hemodynamics (Kennady)	50.9	2 1/4		50.0	2 1/4	50.7	2 1/4
	Nuclide Metabolism (MacDonald)	97.7	4 3/4		67.1	3	68.5	3
	Radiodiagnostic Agent Development (Taplin and MacDonald)	77.2	3		84.9	3	79.7	3
	Clinical Studies-Short Lived Isotopes (Webber)	74.0	3		49.7	2 1/4	51.4	2 1/4
	Biomedical Cyclotron Facility (Cassen and MacDonald)	25.1	3/4		119.6	5	156.6	6 1/2
	Leukemia Biology (Hays)	93.2	4		96.1	4	99.6	4
	Mammalian Radiobiology (Hennessy)	84.4	3		27.4	3/4	27.7	3/4
	ACTIVITY TOTAL	$ 685.0	29	$	708.0	29 1/2	$ 755.0	31
	TOTAL 060000 PROGRAM-	$2,480.0	104	$2,406.0		99	$2,536.0	102

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

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

4. Date Prepared: April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	3 1/4	2	1 1/2
(b) Other Tech.	-	-	-
Total:	3 1/4	2	1 1/2

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 33,000	$ 18,100	$ 18,500
(b) Materials & Services	2,600	2,400	2,400
(c) Indirect Expenses *	17,000	8,300	8,600
Total:	$ 52,600	$ 28,800	$ 29,500

* 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 1970

(1) Zucker, R.M., and Cassen B.: The Separation of Normal Human Leukocytes by Density and Classification by Size. Blood 34, 591-600, 1969.
(2) Zucker, R. M., Fetal Erythroid Cell Development: Size Distributions and Density Gradients. Journal of Cellular Physiology, Vol 1970.

12. SCOPE OF THE PROJECT

In this project studies are being made at the cellular and hematological level by the use of new methods and techniques developed in this laboratory (See Budget Activity 06-02-08).

Applications are being made to the study of density and size groups in populations of leukocytes from treated and untreated leukemia and lymphosarcoma patients. The effects of antilymphocytic drugs are of especial interest. (See 14 below).

The new methods have been applied to the study of the prenatal and neonatal development of hematopoietic cells in mice (See 14 below).

13. RELATIONSHIP TO OTHER PROJECTS

Particularly Dr. E. Cronkite, Brookhaven National Laboratory and Dr. Marvin Van Dilla, Los Alamos. Many other investigators all over the world are rapidly becoming interested in these types of hematological investigation.

14. TECHNICAL PROGRESS IN FY 1970

A paper has been prepared for publication describing the various density and size groups of leukocytes in untreated and treated chronic lymphocytic leukemia, myelocytic leukemia and lymphosarcoma.

Buoyant density centrifugation in bovine serum albumin density gradients demonstrated that lymphocytes can be easily separated from erythrocytes and granulocytes and that the lymphocytes can be further separated into density subgroups. Each density subgroup can be characterized by its own size distribution spectrum by use of the perfected Coulter aperture sensor and multichannel analyzer system. It was found that large size lymphocytes are less dense than the smaller size lymphocytes.

The size varies monotonically with the volume. Typical plots of this variation have been obtained and theoretically analyzed.

Through the courtesy of Dr. James Scott, Chief of Hematology at the Los Angeles Veterans Administration hospital these methods have been applied to blood from treated and untreated leukemia patients.

It was demonstrated that these techniques were useful for the rapid diagnosis of different types of leukemia (chronic lymphocytic, chronic myelocytic, monomyelocytic and lymphoblastic). At least two distinct leukemic populations have been determined by size and separated by density: It was shown that cytoxan, and alkylating agent that is used in chronic lymphocytic leukemia therapy, selectively destroys the small lymphocyte population. Phytoheamagglutinin stimulation of separated chronic leukemic lymphocytes revealed a differential growth response in cell cultures.

A patient diagnosed to have a lymphosarcoma had a small fraction (less than 2%) of his lymphocytes similar to those found in chronic lymphocytic leukemia but in addition had an overwhelming quantity of lymphoblasts. Because of the difference in density between small lymphocytes and cancer lymphoblasts the two populations could be separated.

Density gradients and size distributions can also be used to separate very small quantities of malignant cells for their characterization in cancer diagnosis.

Size and density distributions of erythroid cells from fetal C57BL-6 mice during the 11th through 20th days of gestation are fully described in paper (2) cited in 11 above (Publications During FY 1970). The results suggest that the decrease in size and increase in density of non-nucleated cells observed is due to the release of successively smaller reticulocytes from the liver. The mean volume of nucleated erythroid cells is about ten times the mean adult erythrocyte volume. The gestation age of an embryo can be determined from blood cell size distributions.

15. EXPECTED RESULTS IN FY 1971

It is expected that there will be a continuation of thorough and systematic measurements of changes in lymphocytes 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 erythropoietic stem cell can be concentrated and separated.

It is expected that small numbers of extra large cells can be selected by a Coulter aperture sensor to trigger off submicrosecond flash microholograms of them for developing a system for their identification.

16. EXPECTED RESULTS IN FY 1972

It is anticipated that in FY 1972 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, polypetites, 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 - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	1 3/4	1 1/2	1 1/2
(b) Other Tech.	-	-	-
Total:	1 3/4	1 1/2	1 1/2

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 20,900	$ 16,500	$ 16,500
(b) Materials & Services	7,500	5,400	5,400
(c) Indirect Expenses *	17,000	8,300	8,600
Total:	$ 45,400	$ 30,200	$ 30,500

* 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 1970

Byfield, John E., Young Chang Lee, and Leslie R. Bennett: Thermal Instability of Tetrahymena Ribosomes: Effects on Protein Synthesis, Biochem. Biophys. Res. Comm. 37, (5), 806-812, 1969.

Byfield, J.E., Y. C. Lee, and L. R. Bennett: Bonding of Small Molecules to DNA Following Ionizing Radiation, Nature (in press)

12. SCOPE OF THE PROJECT

Budgetary stringencies require a modest phasing down of research in this area. "Late effects" represent a facet of the general biological area of radiation biology. Research in this section is part of the Laboratory program of Radiation Biology. To an increasing degree work in this section has been seeking the interpretation of late effects at the physiological and molecular level. This shift in emphasis is expected to continue.

Currently the research of this section is concerned with: (1) The mechanisms, at both the physiological and molecular biological level, of the life shortening process following total body irradiation. This is a gradual change in emphasis from earlier studies which were primarily concerned with the pathology and statistics of life shortening, disease incidence, and clinical course. (2) The consequences of partial body irradiation which have not been as clearly defined either as to pathology or life shortening. The study of partial body irradiation represents an area of growing importance since this type of irradiation results from certain toxicological and industrial exposures as well as improperly administered diagnostic x-ray. This study is of increasing importance in nuclear medicine, radiation therapy and organ transplantation.

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 in other institutions have been, or are now, concerned with the delayed effects of irradiation.

14. TECHNICAL PROGRESS IN FY 1970

A study of the effect of heavily labelled antigen on the survival of mice has been completed. Preliminary analysis of this data demonstrated life shortening with doses of 1 mc I-131 labelled Tobacco Mosaic Virus and none at 0.5 mc. This suggested that with quantitites of approximately 0.25 mc, which will produce a marked depression in the immune response, there will not be an associated significant shortening of life span. Using transplantation antigen heavily labelled with I-131 to suppress immunity has produced prolonged survival of skin grafts in mice.

Further studies on salt stress as a means of demonstrating the latent injury of irradiated rats have shown that animals subjected to salt stress have a significantly lower incidence of tumors than do non-stressed animals. This effect of salt stress was also demonstrated for non-irradiated animals.

Following-up studies with DL-glyceraldehyde and Ehrlich ascites tumor cells demonstrated that DL-glyceraldehyde had an additive effect with radiation

and suggested that the mechanism might involve interference with DNA repair. Further support for this view was obtained in in vitro studies on binding of small molecules to irradiated DNA, where amino acids, glyceraldehyde, and other small molecules were demonstrated to form covalent bonds with irradiated DNA.

15. EXPECTED RESULTS IN FY 1971

To date there are no reported studies on the effect of irradiation on mitochondrial DNA. Presumably it is as vulnerable to irradiation injury as is nuclear DNA. This could have an important role in the late somatic effects of irradiation, and might explain in part the reduced capacity of the irradiated animal to perform metabolic work as measured by chronic salt stress. This would be especially true if there is a limited mitochondrial DNA repair mechanism. HELA cell mitochondrial DNA will be studied by isotope labelling and Cesium gradient fractionation to measure the repair capacity of these organelles.

Salt stress experiments will be continued to confirm the tumor inhibiting effect of this treatment, and if possible to relate this to current concepts of tumor genesis.

Extended field irradiation of lymphomas and Hodgkin's disease has greatly improved the cure rates of these diseases. At present this form of treatment is largely limited by the associated depression of the bone marrow. Experiments will be carried out using thermal stimulation and anemia to extend the active marrow space into the normally fatty distal marrow spaces. If such marrow expansion can be produced, its effect on protection of rats and rabbits from the acute lethal effects of central body irradiation and the long-term survival following such treatment will be observed. Marrow space size and distribution will be measured by use of Fe-59 and Tc-99m tracers.

16. EXPECTED RESULTS IN FY 1972

It is planned to continue studies on the relationship of the effectiveness of repair mechanisms of nuclear and mitochondrial DNA to observe late effects following local or total body irradiation. Studies will also be continued on the pathology and survival of animals given central body irradiation following expansion of the bone marrow space.

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.:
06-01-02

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	3 ½	3 ½	3 ½
(b) Other Tech.	½	-	-
Total:	4	3 ½	3 ½

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 66,900	$ 61,000	$ 61,000
(b) Materials & Services	16,500	20,100	20,100
(c) Indirect Expenses *	42,400	33,200	34,300
Total:	$ 125,800	$ 114,300	$ 115,400

* 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 1970

M. W. Seraydarian, E. Sato, M. Savageau, and I. Harary. In Vitro Studies of Beating Heart Cells in Culture XII. The Utilization of ATP and Phosphocreatine in Oligomycin and 2-Deoxyglucose Inhibited Cells. Biochim. Biophys. Acta 180, 264 (1969).

G. A. Langer, E. Sato, and M. W. Seraydarian. Calcium Exchange in a Single Layer of Cardiac Cells Using a Method for Direct Counting of Cellular Ca^{45} Activity. Circ. Res. 24, 589 (1969).

M. W. Seraydarian, E. Sato, and I. Harary. Effect of Inhibitors on the Calcium Exchange of Heart Cells in Tissue Culture. J. Molecular Cardiology. (In press).

M. W. Seraydarian, E. Sato, and I. Harary. In Vitro Studies of Beating Heart Cells in Culture XIII. The Effect of 1-Fluoro-2,4-dinitrobenzene. Arch. of Biochem. and Biophys. (In press).

H. Lewis and I. Harary. In Vitro Studies of Beating Heart Cells in Culture XIV. Reversible Changes in the Myosin Level. Arch. of Biochem. and Biophys. (In press).

12. SCOPE OF THE PROJECT

We are continuing our study of the relation of specific metabolism to specific function using the cultured heart cells as a model. Our project is designed to investigate the control of metabolism as it is designed to bring about specific function and synthesis of specific proteins. As markers of function we use the cells ability to beat, the synthesis of muscle proteins, such as myosin, and the visible subcellular 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 Subcellular 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 1970

Heart muscle cells growing in culture provide a model system for studying development and control of specific functions in tissue. Myosin is a specific marker, and its study could provide much information on control of specific protein synthesis. Myosin was measured by its Ca++-activated ATPase activity. In complete medium (Ham's F10 + 20% serum), heart cells grew and total myosin increased. In basal medium (Ham's F10), there was no growth and total myosin decreased: addition of serum led to a restoration of the myosin level. More detailed studies of myosin synthesis and degradation require use of tracer methods, and isolation of pure myosin. Existing isolation methods (DEAE chromatography; specific precipitation procedures) failed to remove contaminating radioactive proteins. An acrylamide gel electrophoresis procedure was developed and this appeared to remove all contaminating protein, and also permitted the amount of myosin to be estimated. Changes in ATPase activity and in amount of myosin were directly related.

Factors that may affect myosin synthesis are being studied. Purification of the serum factor is being attempted. Various hormones have been tested, and insulin appears to stimulate myosin synthesis in cells on basal medium. As an extension of these studies, uterus cells have been grown in culture, and estradiol has been found to affect growth rate, morphology and enzyme levels.

A study of the control of myosin synthesis also requires a study of the messenger-RNA and polyribosomes involved in its synthesis. Methods of labeling the RNA, of separating RNA species, and of isolating polyribosomes with their associated messenger-RNA and protein have been tested. RNA-DNA hybridization experiments are under way, and it is hoped that studies of synthesis and turnover of myosin-messenger-RNA may be possible.

15. EXPECTED RESULTS IN FY 1971

We expect to study the development of muscle in culture in the following ways:

1. We plan to follow the affect of hormones on myosin in heart, skeletal and uterine muscle, continue the survey of hormones, study the kinetics of those which affect the level of myosin and investigate the mechanism of the effect of triiodothyronine and insulin on maintaining myosin.

2. We plan to fractionate the serum protein to see if we can isolate and identify the factor present in serum which maintains the level of myosin.

16. EXPECTED RESULTS IN FY 1972

The following year we plan mainly to study the control and regulation of myosin in muscle cells. This requires investigation of the turnover of myosin under various physiological conditions and the initiation and synthesis of myosin and messenger RNA for myosin.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title:
Macromolecular Chemistry

3. AEC Budget Activity No.:
06-01-02

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	2	-	2
(b) Other Tech.	1	½	1
Total:	3	½	3

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 25,400	$ 3,700	$ 27,900
(b) Materials & Services	12,100	3,200	8,600
(c) Indirect Expenses *	17,000	8,300	17,200
Total:	$ 54,500	$ 15,200	$ 53,700

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

11. PUBLICATIONS DURING FY 1970

NONE

12. SCOPE OF THE PROJECT

Investigations into the relationship between structure and function in biological high polymers, such as the nucleic acids and proteins (structural, enzymic and hormonal) as well as small cytoplasmic particles and viruses, has assumed a most important role in biological research. The biological function of most macromolecules is largely dependent 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 numberous 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 La Jolla (Singer) etc.

14. TECHNICAL PROGRESS IN FY 1970

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.

15. EXPECTED RESULTS IN FY 1971

Dr. Norman S. Simmons will be on sabbatical leave during FY 1971, but will return to the Laboratory beginning FY 1972.

16. EXPECTED RESULTS IN FY 1972

It is expected that the conformational analysis of macromolecules by circular dichroism, 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.:
06-01-02

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	3 ½	3	3
(b) Other Tech.	½	1	1
Total:	4	4	4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 37,900	$ 43,400	$ 44,500
(b) Materials & Services	4,800	3,700	3,700
(c) Indirect Expenses *	25,400	24,900	25,700
Total:	$ 68,100	$ 72,000	$ 73,900

* 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 1970

J. F. Mead. Phylogenetic Relationships in Lipid Metabolism. J. Am. Oil Chem. Soc. 46, 32 (1969) (Abstract).

B. J. Burns, R. B. Alfin-Slater, and J. F. Mead. Effect of Dietary Sterculic Acid on the Lipid Composition of Rat Tissues. J. Am. Oil Chem. Soc. 46, 62 (1969) (Abstract).

J. F. Mead and D. F. Haggerty. Some Relationships of Polyunsaturated Fatty Acid Metabolism to Atherosclerosis. J. Am. Oil Chem. Soc. 46, 94 (1969) (Abstract).

K. Lippel and J. F. Mead. Alpha Oxidation of 2-Hydroxy Tetracosanoate in the Rat. Lipids 4, 129 (1969).

O. A. Schjeide, R. I-San Lin, E. A. Grellert, F. R. Galey, and J. F. Mead. Isolation and Preliminary Chemical Analysis of Coated Vesicles from Chicken Oocytes. Physiol. Chem. and Physics. 1, 141 (1969).

G. A. Dhopeshwarkar, R. Maier, and J. F. Mead. Incorporation of Acetate-1-^{14}C into the Fatty Acids of the Developing Rat Brain. Biochim. Biophys. Acta 187, 6 (1969).

B. J. Burns, M. E. Fewster, and J. F. Mead. Quantitative Densitometric Thin Layer Chromatography of Lipids Using Copper Acetate Reagent. J. Chromatography 43, 120 (1969).

M. E. Fewster and J. F. Mead. Lipid Composition of Glial Cells Isolated from Bovine White Matter. Abs. of 2nd. Int. Meeting of Int. Soc. of Neurochem., p. 41 (1969).

F. Wolfgram, M. E. Fewster, and J. F. Mead. The Amino Acids and Lipids of Myelin in Multiple Sclerosis. Abs. of 2nd Int. Meeting of Int. Soc. of Neurochem., p. 61 (1969).

G. A. Dhopeshwarkar and J. F. Mead. Fatty Acid Uptake by the Brain. II. Incorporation of [1-^{14}C] Palmitic Acid into the Adult Rat Brain. Biochim. Biophys. Acta 187, 461 (1969).

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 work 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

Investigators in this field form a rather close-knit group who continuously exchange views, engage in joint research, furnish information and chemicals and often work in each other's laboratories. Those related particularly to my work are the following:

This Laboratory

Dr. A. J. Fulco (Biosynthetic Control), Dr. I. Harary (Enzyme Chemistry), Dr. D. R. Howton (Organic Chemistry), Dr. J. Nevenzel (Tracer Synthesis), Dr. H. Herschman (Developmental Biology), Dr. J. de Vellis (Developmental Radiobiology).

This University

Dr. A. L. Barber (Peroxide Effects), Dr. M. Gordon (Marine Biology), Dr. J. Nyc (Phospholipid Function), Dr. R. B. Alfin-Slater (Lipid Nutrition), Dr. M. Schotz (Veterans Administration – Adipose Tissue Metabolism), Dr. G. Popjak (Sterol Metabolism), Dr. S. Eiduson (Neurochemistry), Dr. F. Wolfgram (Neurochemistry), Dr. F. Adams (Pediatrics - Lung Lipids), Dr. J. H. Menkes (Pediatrics - Brain Lipids).

AEC Laboratories and Grants

Dr. F. L. Snyder (O.R.A.U. – Plasmalogen Metabolism), Dr. W. R. Cornatzer (U. North Dakota – Phospholipid Metabolism), P. D. Klein (Argonne – Sterol Metabolism).

U.S.A.

Dr. A. A. Benson (U.C.S.D. – Membrane Lipids), Dr. J. S. O'Brien (U.C.S.D. – Brain Lipids and Diseases), Dr. P. K. Stumpf (U.C.D. – Plant Lipids), Dr. A. L. Tappel (U.C.D. – Peroxides and Aging), Dr. R. L. Havel (U.C.S.F. – Blood Lipids), Dr. D. J. Hanahan (U. Arizona – Membrane Lipids), Dr. R. O. Brady (N.I.H. – Brain Lipids and Diseases), Dr. G. Rouser (City of Hope – Analytical Methods), Dr. L. A. Horrocks (Ohio State – Brain Phospholipids), Dr. R. T. Holman (Hormel Institute – Polyunsaturated Fatty Acids), Dr. H. Schlenk (Hormel Institute – Polyunsaturated Fatty Acids), Dr. W. O. Lundberg

(Hormel Institute – Fatty Acid Nutrition), Dr. K. Bloch (Harvard University – Lipid Metabolism), Dr. R. M. Burton (Washington University – Brain Lipids), Dr. P. R. Vagelos (Washington University – Fatty Acid Metabolism), Dr. N. L. Radin (U. of Michigan – Brain Fatty Acids), Dr. R. H. Furman (Oklahoma Med. Res. – Blood Lipids), Dr. D. Harman (U. Nebraska – Peroxides and Aging), Dr. D. Kritchevsky (Wistar Inst. – Lipids in Cell Cultures), Dr. F. A. Kummerow (U. Illinois – Lipid Metabolism), Dr. F. Mattson (Procter and Gamble – Lipid Digestion), Dr. D. Malins (Bureau Comm. Fisheries – Marine Lipids), Dr. O. Privett (Hormel Institute – Analytical Techniques), Dr. R. Reiser (Texas A and M – Fatty Acid Metabolism), Dr. S. J. Wakil (Duke University – Fatty Acid Biosynthesis), Dr. J. G. Coniglio (Vanderbilt U. – Essential Fatty Acids).

Foreign

Greece (Dr. C. J. Miras, Dr. G. M. Levis), France (Drs. G. and J. Clément, Dr. N. Baumann), Germany (Dr. E. Klenk, Dr. W. Stoffel, Dr. H. Debuch), Czechoslovakia (Dr. Z. Placer), Italy (Dr. R. Paoletti), Israel (Dr. S. Gatt, Dr. Y. Stein), England (Dr. R. B. Bickerstaffe, Dr. A. N. Davison), Japan (Dr. M. Kayama, Dr. M. Uchiyama).

14. TECHNICAL PROGRESS IN 1970

Further progress in the elucidation of the alpha-oxidation system of brain has involved the use of C_{24} substrates (as α-hydroxy-tetracosanoic acid) rather than the more readily available C_{18} analogs used previously. This has involved considerable effort in solubilization of this very insoluble substrate in an attempt to get it into contact with the enzyme protein. Success has been achieved with the use of a monoglyceride-bile salt micelle and α-oxidation takes place readily with brain homogenates in the presence of ATP, ferrous ion and NADPH. In a parallel study with Dr. Shimon Gatt of the Hadassah Medical School, Israel, it was found that the liver C_{16} alpha-oxidation system does not require the substrate to be in micellar form and that cofactor requirements are quite different.

The passage of fatty acids across the blood-brain barrier has been shown to occur readily with 1-^{14}C palmitate and 1-^{14}C oleate injected or administered orally. These fatty acids appear to be incorporated into brain cell lipids as rapidly and completely as is acetate.

Further investigation of the metabolism of polyunsaturated fatty acids in cells in culture has taken advantage of the availability of a liver cell line. In suspension culture these cells are incapable of performing the desaturation steps between linoleate and arachidonate whereas in monolayer, they appear to do this readily. In monolayer, they have a high phospholipid content and are much more extended, indicating a larger proportion of membrane per cell.

15. EXPECTED RESULTS IN FY 1971

During this year further advances will be made in elucidating the mechanism of alpha-oxidation of long-chain fatty acids. The function of oligodendrocytes (and mitochondria derived therefrom) in the elongation of the brain fatty acids will be investigated and the mechanism of regulation of this function will be considered.

The passage of linoleate-1-^{14}C and linolenate-1-^{14}C across the blood-brain barrier will be studied and an attempt will be made to delineate the site of discrimination for linolenate-derived fatty acids in brain lipids.

The mechanism of loss of desaturation ability in certain cells in culture should be elucidated in liver cells in different types of culture and the function of the polyunsaturated fatty acids in the membrane lipids will be investigated.

16. EXPECTED RESULTS IN FY 1972

Future research will naturally depend on the results obtained from current efforts. Nevertheless, it can be anticipated that by the end of fiscal 1972, we will have achieved a greater knowledge of the processes contributing to the development and aging of the brain, to the establishment and the nature of the blood-brain barrier, to the function of certain enzymatic reactions in the brain and to the role of polyunsaturated fatty acids in cell membranes. Such goals, though ambitious, are realizable and will be well worth the time and effort spent on them.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title:
Organic Chemistry

3. AEC Budget Activity No.:
06-01-02

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA

7. Person in Charge:
David R. Howton

8. Project Term:
From: 1959 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
Total:	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 40,300	$ 43,400	$ 46,700
(b) Materials & Services	5,600	4,700	4,700
(c) Indirect Expenses *	25,400	24,900	25,700
Total:	$ 71,300	$ 73,000	$ 77,100

* 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 1970

D. R. Howton and R. A. Stein. Ahmad-Strong Synthesis of 8-, 9- and 10-Pentadecynoic Acids. J. Lipid Res. 10, 631-635 (1969).

D. R. Howton. Empirical Melting Point-Structure Relationships in the Normal Acetylenic Carboxylic Acids. J. Chem. Soc. (London), Series B, 184-188 (1970).

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 phenomena and of chemical degradation procedures will, of course, be directly pertinent.

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

13. RELATIONSHIP TO OTHER PROJECTS

Los Alamos Scientific Laboratory (H. W. Langham): "Behavior of Cell Membrane Mechanisms During and Following Gamma Radiation"; 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.); Dept. of Food Science and Technology, Univ. of Massachusetts (W. W. Nawar).

14. TECHNICAL PROGRESS IN FY 1970

Present views of the radiolysis of saturated carboxylic ("fatty") acids by low LET radiation (e.g., $Co^{60}\gamma$) hold that the principal primary change

involves decarboxylation, yielding CO_2 and the corresponding saturated hydrocarbon, via a mechanism (or mechanisms) not yet understood in detail. Burr's studies (J. Phys. Chem. 61, 1481 (1957)) with deuterium-labeled acetic acid suggest strongly, however, that the reaction cannot be considered "molecular" (i.e., RCOOH $\rightsquigarrow$ RH + CO_2), and thus that formation of the hydrocarbon probably occurs via the intermediacy of a primary alkyl free radical (R.) which acquires a hydrogen atom from a variety (depending on the structural complexity of the fatty acid involved) of sites, among which that of the hydrogen of the carboxyl group is apparently least likely. On the other hand, abstraction of hydrogen from any of the methylene groups of a straight-chain saturated fatty acid should be energetically favorable (from that adjacent to the carboxyl group most so), leading ultimately to formation of dehydropolymeric products, presumably linked for the most part at carbon adjacent (α) to the carboxyl group.

That the polymeric material formed by γ-irradiation of stearic acid is indeed α-linked has been demonstrated on the basis of the fact that the major dimeric component exhibits gas-chromatographic behavior identical with that of the slightly slower-moving of the pair (presumably dl and meso diastereomers) of dehydrodimers produced by thermal decomposition of di-t-butylperoxide in methyl stearate. (This reaction, presumably because of the high temperature at which it is carried out — 130 - 40° — yields almost equal amounts of the two α-linked isomers, together with a small amount of material believed to be cross-linked elsewhere, which precedes the others in glc.) The γ-irradiation product also contains small amounts of the second α-linked and of the elsewhere-linked compounds. That the major products of the peroxide pyrolysis reaction are α-linked was shown by Wheeler et al. (J. Am. Oil Chem. Soc., 42, 2 (1965)) by direct comparison of a crystalline derivative with that obtained by unequivocal classical synthesis, although the assignment of configuration (dl vs. meso) remained unsettled. Study of the properties of a number of lower-homologous dl and meso pairs of such α,α'-disubstituted succinates by Eberson (Acta Chem. Scand. 20, 739 (1966)) has shown that the meso isomers are more strongly retained in gas chromatography involving non-polar liquid phases and less strongly adsorbed in chromatography on columns of H-bond-contributing adsorbents (e.g., silicic acid). On this basis, it now seems clear that the principal γ-irradiation product has the meso configuration. (Eberson's apparent belief that action of concentrated sulfuric acid converts the dl - but not the meso - ester to the corresponding anhydride, and affects dl-meso isomerization is not in accord with our observations, which are being extended currently.)

Since the detailed crystal structure of stearic acid is known (from x-ray diffraction analysis), our present ideas concerning the course of radiolysis of this substance anticipates that samples irradiated in the solid state should contain trapped n-heptadecyl and/or α-carboxyl radicals (depending on whether or not the initially-formed alkyl radicals possess sufficient mobility within the crystal lattice to permit abstraction of an α-methylene hydrogen atom from an adjacent molecule of the starting material). Such material does indeed exhibit a strong, anisotropic electron-spin-resonance spectrum (apparently indefinitely stable at temperatures below its melting point), detailed analysis of which should reveal the nature of the radical(s) involved. The observation that dissolving irradiated crystalline stearic acid in a solution of iodine in benzene yields neither heptadecyl iodide nor α-iodostearic acid suggests 1) that the material contains no heptadecyl radicals; and 2) that α-carboxyl radicals do not react readily with elemental iodine; these preliminary correlations are being investigated further.

The mechanism we have proposed for decarboxylation of fatty acids irradiated in the condensed state (see Howton and Wu, J. Am. Chem. Soc., 89, 523 (1967)) implies that the molecule initially ionized remains intact, while that to which it is hydrogen-bonded actually suffers decarboxylation. Evidence in support of this theory is being sought by determination of the relative amounts of different hydrocarbons produced by irradiation of mixtures of fatty acids, the extensiveness of decarboxylation of which per unit dose of irradiation is known to be inversely proportional to chain length. Observation that the ratio of C_{15} to C_{17} hydrocarbon obtained on irradiation of a mixture of equimolal quantities of crystals of palmitic and stearic acids (1.065) is increased to 1.173 if the substances are melted together (and thus presumably contain the statistically-expected 50% content of mixed palmitic-stearic "dimers") prior to irradiation provides tentative support for the proposed mechanism. Mixtures of palmitic and oleic acid (for which $G_{hydrocarbon}$ are widely disparate and should thus evidence the predicted effect still more clearly) are to be studied similarly.

15. EXPECTED RESULTS IN FY 1971

Providing investigations in progress are brought to satisfactory conclusion, it is anticipated that our attention will turn next to exploring a developing viewpoint that changes produced in a lipid molecule on exposure to ionizing radiation should reveal its immediate molecular environment. We plan presently to study the radiolysis of crystalline saturated triglycerides (the crystal-structure of such substances being known in detail) in hopes of being able to demonstrate creation of intramolecular cross-linkage joining the juxtaposed 1- and 3-fatty acyl hydrocarbon chains. It appears reasonably probable that intramolecularly cross-linked triglycerides should have essentially the same silicic-acid-chromatographic behavior as that of unaltered material, while intermolecularly cross-linked dimeric triglycerides should be significantly more strongly adsorbed and thus separable from the other type of product. To the extent that the "triglyceride" fraction contains cross-linked material, transesterification with acidified methanol should yield dehydrodimers of methyl stearate, chromatographic characteristics of which (in distinction to those of the accompanying methyl stearate, stemming from unaltered triglyceride) are now well established.

16. EXPECTED RESULTS IN FY 1972

These studies should lay a sound groundwork for those envisaged from the outset - the attempt to reveal the disposition of amphipathic lipids in membranes. From this somewhat remote vantage point it would appear reasonable to anticipate that initial studies would involve determination of alteration in the fatty acid spectra derived from both the α- and β-positions of an adequately characterized natural lecithin (or other type of phospholipid) isolated by chromatographic techniques from (for example) egg yolk, following exposure to Co^{60} γ-irradiation. Of particular interest will be determination of influence of the state of the substance at the time of irradiation - crystalline, and in various fairly well-understood states of dispersion produced in the presence of different amounts of water. With information gained by such studies in hand, we should then be prepared to consider with some hope of rational interpretation the significance of lipid alteration arising from γ-irradiation of natural membranous and other lipoprotein materials, such as erythrocytes (intact and ghost), serum lipoproteins of various classes, outer and intracellular membranes, and mylein.

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.:
06-01-02

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	1	1	1
(b) Other Tech.	½	1	1
Total:	1 ½	2	2

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 18,600	$ 25,700	$ 25,700
(b) Materials & Services	2,200	3,100	3,100
(c) Indirect Expenses *	8,500	16,600	17,200
Total:	$ 29,300	$ 45,400	$ 46,000

* 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 1970

A. H. Banner, J. C. Nevenzel, and W. R. Hudgins. Studies in Marine Toxins IV. The Contamination of Wake Island Lagoon. Atoll Res. Bull., No. 122, March 30, 1969.

M. Kayama, Y. Tsuchiya, and J. C. Nevenzel. The Hydrocarbons of Shark Liver Oils. Nippon Suisan Gakkaishi [Bull. Japan Soc. Sci. Fisheries], 35, 653-64 (1969).

J. C. Nevenzel, W. Rodegker, J. S. Robinson, and M. Kayama. The Lipids of Some Lantern Fishes (Family Myctophidae). Comp. Biochem. Physiol., 31, 25-36 (1969).

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 unsaturated fatty acids have been labelled with carbon-14 or tritium by techniques developed in this Division.

A second field of interest is the metabolism of the non-glycerol lipids (i.e., the waxes) with particular emphasis on their biogenesis in marine animals and higher plants. A survey of the occurrence and composition of the waxes in various species is of continuing interest, and necessarily has involved the preliminary development and testing of methods for the extraction, analysis, and separation of wax 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. In the second phase, carbon-14 or tritium labelled substrates have been used to trace the pathways involved in the biosynthesis of the wax constituents, and to study their metabolism. Techniques will be developed for the controlled chemical degradation of labelled waxes.

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? Lipid biochemistry provides a promising tool for the study of developmental biology in some of the fish species investigated, since the main lipid types are different in the adults and in the eggs (where lipid is the main reserve energy store). We hope 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 University of Glasgow (G. Eglinton), Laboratoire de Biologie Vetetale, Bellvue, France, (P. Mazliak), and Agriculture Research Service, USDA Beltsville, Md. (W. A. Gentner), Department of Biochemistry, Washington State Univ., Pullman, Wash. (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 1970

Again, most of the year was spent at Scripps Institution of Oceanography, La Jolla, Calif. With Mr. Richard F. Lee (Marine Biology Research Division) and Dr. G.-A. Paffenhöfer of the Food Chain Research Group of the Institute of Marine Resources [supported by AEC Contract AT(11-1)GEN-10, P.A.-20], we have followed the lipid changes along a short marine food chain consisting of unicellular diatoms or dinoflagellates to the copepod, Calanus helgolandicus to anchovies or sardines. The copepods contain high amounts of total lipid (12.5 - 70%, dry wt.) of which up to 80% may be wax esters, but the diatoms, dinoflagellates, and fishes of this food chain do not contain wax esters. During starvation the wax esters in copepods decrease at least proportionately to the loss of total lipids, and there are dramatic changes toward shorter and more saturated esters. Turnover studies in copepods fed on ^{14}C-labelled diatoms showed that the total radioactivity built up rapidly in the wax ester fraction and then decreased on either subsequent feeding with unlabelled diatoms or starvation. From these data we conclude that the wax esters are primarily an energy reserve in these epipelagic copepods. Two papers describing this work are in press. In order to metabolize dietary wax esters, anchovies and sardines presumably have active wax lipases and long-chain alcohol dehydrogenases; in vitro studies with Mrs. E. A. Baker have confirmed the conversion of hexadecanol-1-^{14}C into fatty acids in liver and red muscle slices.

With Mr. Lee and Jed Hirota we are surveying the relations between depth of occurrence, lipid content, proportion of wax esters, and homolog patterns of the wax esters in copepod species of the open sea from the surface to 3,000 meters. Preliminary conclusions from thirty species are that both the total lipid content and the proportion of wax esters increase with increasing depth. Other variables which probably affect the observed differences are temperature, season, sex, and taxonomy, but we are not yet able to assess their separate contributions. The wax ester homologs present do not seem to correlate with depth, but rather for all species consist of C_{30}-C_{42} components with maxima in the C_{34}-C_{38} range. This work will be presented at a Symposium on Oceanic Biochemistry, during the Northwest Regional Meeting of the American Chemical Society in Seattle, Wash., on June 19, 1970.

We anticipate that wax ester lipases are present in the muscle of those lantern fishes which contain major amounts of this lipid type, and during a month's cruise on the R/V Alpha Helix we will attempt to isolate and characterize such an enzyme, using the liberation of radioactive fatty acid from a synthetic ^{3}H-labelled wax ester as a sensitive assay.

15. EXPECTED RESULTS IN FY 1971

A survey by thin-layer chromatography of the lipids from a large number of algae and protozoans has brought to light several species which seem to

contain wax esters and can be maintained in laboratory culture, thus permitting more sophisticated investigations of wax ester biosynthesis in a marine organism. Possible candidates are Isochrysis galbana (Haptophyceae), Oxyrrhis marina (a heterotrophic dinoflagellate), and two Eutreptiella sp. (marine euglenoids). With such cultures available we expect to investigate the interconversions of fatty acids and long-chain alcohols to learn more about possible intermediates, cofactors, and the regulation of alcohol and wax ester biosynthesis. In addition, many details of the pathways by which these interconversions are achieved in fish muscle and liver will be available when analyses are completed of the lipid samples obtained by Dr. M. Kayama in This Laboratory in 1966-67 while studying the incorporation of various precursors (acetate, palmitate, oleate, 1-hexadecanol) into wax esters in lantern fishes. Similar data will be obtained from the fish liver and red muscle experiments with hexadecanol-1-^{14}C.

16. EXPECTED RESULTS IN FY 1972

Studies of the enzymes involved in the interconversion of fatty acids and alcohols will continue. The effect of temperature on these reactions and on the fatty acid composition of the wax esters and other lipid types present in the unicellular experimental organisms will be studied.

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.:
06-01-01

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA and Gulf General Atomics Incorporated, San Diego, Calif.

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

8. Project Term:
From: 1947 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	4 ½	4 ½	4 ½
(b) Other Tech.	½	½	½
Total:	5	5	5

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 60,700	$ 66,000	$ 69,300
(b) Materials & Services	19,600	19,700	19,700
(c) Indirect Expenses *	33,900	33,200	34,300
Total:	$ 114,200	$ 118,900	$ 123,300

* 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 1970

Zimbrick, J. D., and L. S. Myers, Jr.: An Electron Paramagnetic Resonance Study of Irradiated Frozen Aqueous Sugar Solutions. Radiation Research 39, 509 (1969) (Abstract).

Al-Thannon, A. A., and L. S. Myers, Jr.: Pulse Radiolysis of Cysteine, Cystine, and DNA. Radiation Research 39, 514 (1969) (Abstract).

Zimbrick, J. D., J. F. Ward, and L. S. Myers, Jr.: Studies on the Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA. I. Pulse and Steady State Radiolysis of 5-Bromouracil and Thymine. Int. J. Radiat. Biol. 16, 505-523 (1969).

Zimbrick, J. D., J. F. Ward, and L. S. Myers, Jr.: Studies on the Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA. II. Pulse and Steady State Radiolysis of Regular and Bromouracil-Substituted DNA. Int. J. Radiat. Biol. 16, 525-534 (1969).

Myers, L. S., Jr., and L. M. Theard: Pulse Radiolysis of Nucleic Acid Constituents and Related Compounds. I. Optical Spectrum and Reactivity of the 5,6-dihydrothyminyl Free Radical. J. Am. Chem. Soc. (Accepted).

Myers, L. S., Jr., Aida Warnick, Mary Lynn Hollis, J. D. Zimbrick, L. M. Theard, and F. C. Peterson: Pulse Radiolysis of Nucleic Acid Constituents and Related Compounds. I. Resolution of a Rapidly Decaying Transient Absorption in Cytosine Solutions. J. Am. Chem. Soc. (Accepted).

Myers, L. S., Jr., Mary Lynn Hollis, L. M. Theard, F. C. Peterson, and Aida Warnick: Pulse Radiolysis of Nucleic Acid Constituents and Related Compounds. III. Optical Spectra and Reactivity of Organic Free Radicals Formed by Reaction of Hydroxyl Free Radical with Pyrimidine Bases. J. Am. Chem. Soc. (Accepted).

12. SCOPE OF THE PROJECT

The purpose of this work is to gain increased information about the initial radiation induced reactions which are responsible for the biological effects of radiation, and to develop means of modifying these reactions. The reactions considered are the ones caused by substances produced at each site at which radiation deposits energy (ionized molecules, excited molecules, and electrons), and by substances formed from them within 10^{-9} seconds after energy deposition (organic free radicals, charged organic radical ions, hydrogen atoms, hydroxyl free radicals, and solvated electrons). In cellular fluids or other aqueous media these are exceptionally reactive substances, and within a millisecond after energy deposition they undergo various reactions with one another and with other molecules. These reactions are the subject of our investigations. Their direct observation requires a technique which permits measurements in less than a millisecond, pulse radiolysis. Essential information about them can also be obtained by electron paramagnetic resonance spectroscopy (EPR) of irradiated model systems in which one or another of the usually reactive species is trapped, such as in solids or frozen aqueous solutions, and from results of conventional radiation chemistry and biochemistry. Principal emphasis at present is on reactions of DNA or DNA constituents because considerable evidence suggests that damage of DNA by radiation is a major cause of radiation effects in living cells. The results of this work will lead to a better understanding of the biological effects of radiation, and will provide a rational base for development of means of protecting against radiation effects and of using radiation effects in beneficial ways such as in cancer therapy.

This section is also responsible for the Laboratory's Radiation Facility.

13. RELATIONSHIP TO OTHER PROJECTS

This work is closely related to investigations in this Laboratory under J. F. Ward, D. R. Howton, and E. H. Strickland, and generally so to many others. Laboratories throughout the world, including the Soviet Union, are conducting studies in radiation chemistry and biochemistry. Those most closely related are: (Pulse radiolysis) J. W. Hunt, University of Toronto, Canada; G. Scholes and J. Weiss, University of Newcastle-upon-Tyne, England; G. E. Adams, Mount Vernon Hospital, England; L. M. Theard, Gulf General Atomic Incorporated, San Diego, California; E. Hayon, U. S. Army Natick Laboratories, Natick, Massachusetts; (EPR) J. D. Zimbrick, University of Kansas, Lawrence, Kansas; A. Müller, Karlsruhe, Germany; W. Snipes, Pennsylvania State University, University Park, Pennsylvania; W. Gordy, Duke University, Durham, North Carolina; S. J. Wyard, Guy's Hospital Medical School, London, England; (general radiation biochemistry) B. Ekert, Radium Institute, Paris, France; and W. Garrison, University of California, Berkeley, California.

14. TECHNICAL PROGRESS IN FY 1970

Pulse Radiolysis of Pyrimidine Bases: Interpretation of pulse radiolysis studies of the reactions between the products of water radiolysis (H· (hydrogen atoms), e^-_{aq} (hydrated electrons), and OH· (hydroxyl free radical)) and DNA is impossible without information about the reactions between these substances and DNA constituents. Accordingly an extensive study of the pulse radiolysis of pyrimidine bases in aqueous solution has been carried out. Solutions were exposed to 10 to 20 nanosecond pulses of 10 mev electrons (~1,000 rads), and optical absorption spectra of resulting pyrimidine base free radicals, and decay of absorbance were determined by kinetic spectroscopy. Decay was complete within a millisecond, and some reactions occurred within ten microseconds. The results are being published in a group of three papers. The first deals with the assignment of a transient optical absorption spectrum to a specific free radical structure. The assignment is based on comparison of spectra obtained 1) by abstraction of H· from dihydrothymine by H· and OH·, 2) by addition of H· to thymine, and 3) by addition of e^-_{aq} to thymine, followed by a protonation reaction. The results show that reactions 1) and 2) give nearly identical spectra which are very probably associated with the 5,6-dihydrothymin-5-yl free radical. Reaction 3) gives a different spectrum and product. The second paper reports an analysis of a rapid change in spectral shape following reaction of cytosine with the OH· free radical. Results show that the radical formed initially by addition of OH· to cytosine is unstable, and reacts with OH^- ion to form an anion radical. This analysis provides the key which permits analysis of the data provided in the third paper. This latter paper shows that four different kinds of radicals are formed by reaction of pyrimidine bases with OH· free radical, assigns spectra to the radicals, suggests structures for the radicals, gives conditions for their formation, and discusses briefly their decay. All pulse radiolysis experiments were carried out in cooperation with Dr. L. M. Theard, and were performed at Gulf General Atomic Incorporated, San Diego, California.

Pulse Radiolysis of DNA: The above work, combined with earlier work on the reaction of OH· with deoxyribose and deoxyribose phosphate has made it worthwhile to conduct pulse radiolysis experiments with DNA. Dilute aqueous solutions of DNA have been exposed to short pulses of radiation under con-

ditions such that OH· free radical is the only attacking species. The rate constant for the reaction between OH· and DNA, based on a mean nucleotide molecular weight of 350, is about one-tenth that for reaction of OH· with pyrimidine bases and many other organic compounds. This suggests that OH· will attack DNA in a living cell only if it is formed close to the DNA. The DNA· radical decays much more slowly by radical-radical reactions than radicals formed by smaller molecules, indicating that there is an excellent chance that DNA· radicals will undergo secondary reactions with cellular constituents. The spectrum of DNA· radicals is similar in shape to both the spectrum of radicals formed by reaction of OH· with deoxyribose, and the spectrum of the weighted mean (weighted according to the proportion in DNA) of the spectra given by reaction of OH· with the relevant nucleotides. The DNA· spectrum is about four times as intense as the deoxyribose radical spectrum, and about one-fourth as intense as the mean nucleotide spectrum. These results are consistent with the following conclusions: a) a major proportion of the attack by OH· on DNA is reaction with the bases, b) the DNA· free radicals show substantial hypochromicity, and c) considerable attack occurs on the deoxyribose moiety as well. The shapes of DNA· radical spectra are the same for E. Coli, calf thymus, and salmon sperm DNA, and for native and denatured DNA. The spectrum of radicals formed from denatured DNA is more intense than that given by native DNA. This latter observation is consistent with less hypochromism and more attack on the bases of denatured than of native DNA.

The Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA: It is well known that substitution of 5-bromouracil for thymine in DNA increases the sensitivity of viruses, bacteria, and mammalian cells to radiation. We have reported a study of the mechanism of the sensitization in two papers. The first covers a study of the radiation chemistry of 5-bromouracil in aqueous solutions, using conventional radiation chemistry techniques and pulse radiolysis. The second reports an attempt to apply the knowledge so gained to the radiation destruction of 5-bromouracil substituted DNA isolated from E. Coli. The results show that the reaction of OH· free radical with 5-bromouracil (BU) is similar to that with thymine (T). The reaction of hydrated electrons (e^-_{aq}) with BU proceeds at the same rate as with T, but the chemical nature and reactivity of the products are different. The e^-_{aq} adds to T, to give a stable anion radical, but reacts with BU by dissociative electron attachment to give a highly reactive uracilyl radical. This radical can abstract hydrogen atoms from other organic molecules, such as sugars, to form uracil and an organic free radical. BU reacts readily with ion radicals such as $\cdot CO_2^-$ to give the uracilyl radical, and also with organic free radicals. Experiments with BU-labeled DNA are consistent with a sensitization mechanism based on reactions of the BU moiety with e^-_{aq} and organic free radicals, giving a uracilyl radical which abstracts H· from a neighboring deoxyribose moiety to give a uracil moiety and a sugar radical. The latter can lead to a main chain break. These studies were done in cooperation with Drs. J. D. Zimbrick (AEC Postdoctoral Fellow) and J. F. Ward (Sub-Cellular Radiobiology Section).

The Gamma-Radiolysis of Frozen Aqueous Thymine Solutions: This study was undertaken to determine the products formed on irradiation of T in frozen aqueous solutions, to compare them with products formed in liquid solution, and to determine the mechanisms of the reactions. One of the specific goals was to check an earlier report that a low steady state concentration of thymine dimer is formed in irradiated ices. Different products might be expected in ice than in liquid solutions in part because the thymine is probably distributed heterogeneously in "puddles" of very high thymine

concentration. Reactions involving two or more thymine molecules are more probable in such puddles than in solution. In addition the electron species is undoubtedly not a hydrated electron, and thus its chemical behavior may be different than is observed in liquid solution. Quantitative differences in products formed in ice and liquid water might also be expected because the proportions of e^-, H· and OH· reacting with thymine are probably different and temperature dependent (the e^- is mobile at liquid N_2 temperatures, but OH· becomes mobile only at much higher temperatures). Experiments are in progress to determine 1) dependence of thymine disappearance yields on irradiation conditions and post-irradiation treatment, 2) nature of products and product yields, and 3) formation of free radicals and their reactions as the temperature is increased from 77°K to the melting point. These latter studies are being done by EPR.

EPR Studies of Irradiated Single Crystals of 5-Methylcytosine and Orotic Acid: EPR measurements of irradiated polycrystalline substances give useful information on the yields of organic free radicals, and, in favorable cases, about radical structure. If several radicals are formed, however, their identification requires the more definitive measurements which can be made with single crystals.

As part of a program of studying the formation of free radicals in pyrimidine bases and the structural factors which determine localization of radical sites, an analysis of EPR spectra obtained from irradiated single crystals of 5-methylcytosine and of orotic acid is in progress. Results will be compared with earlier work with other compounds. This work is being done in cooperation with Drs. J. Hüttermann (Postdoctoral Scholar), and J. F. Ward (Sub-Cellular Radiobiology Section).

Other studies: The following are being investigated: a) Mechanisms by which ionizing radiation splits the thymine dimer, and b) Nature and properties of electrons trapped in frozen aqueous solutions (with J. D. Zimbrick).

15. EXPECTED RESULTS IN FY 1971

Investigations of the radiolysis of nucleic acids and their constituents will be continued. In general, pulse radiolysis, EPR spectrometry, and conventional radiation chemical techniques will be used to study the mechanisms of both the direct and indirect action of radiation on DNA constituents, DNA and nucleoproteins. Specific projects follow:

Pulse Radiolysis Study of the Reaction of OH· Free Radical with DNA: The experimental aspects of this work are believed to be nearly complete. It is hoped that analysis of the data will definitely determine the site(s) of attack by OH· on DNA and provide definitive information on the kinetics of DNA· radical formation, stability, and decay.

ESR Study of Free Radical in Irradiated Single Crystals: Determination of the structures of free radicals formed in 5-methylcytosine and orotic acid by irradiation will be completed, and further measurements on other substances will be done if needed to clarify interpretation of the data. The results should improve our understanding of the mechanisms by which radiation damages DNA when it is absorbed directly in the organic molecule.

ESR Study of Trapped Electrons in Frozen Aqueous Sugar Solutions: This work will give insight into the behavior of electrons in irradiated media, and information about the reactions of OH· free radicals with sugars.

Radiolysis of Frozen Aqueous Solutions of DNA Constituents and DNA: Results to date with thymine suggest that investigation of this system is well worth while since it gives information on reactions of the solute with electrons and OH·. We plan to extend the work to include other nucleic acid constituents, DNA, RNA, and nucleoprotein. The information obtained with thymine will facilitate these studies greatly.

Mechanisms by which Ionizing Radiation Splits the Thymine Dimer: A pyrimidine dimer which is readily soluble in water has been synthesized. We hope that with the aid of this compound we can carry out critical experiments which will enable us to determine which substance in irradiated water (H·, OH·, e^-_{aq}, or e^-) splits the dimer, and the mechanism by which it acts.

Pulse Radiolysis of Aqueous Solutions Containing DNA plus a Second Reactive Compound: Modification of Effects of OH· Free Radical on DNA: Results of previous work with simple systems permit us to perform experiments with more complicated systems than used thus far. We plan, for example, to use the pulse radiolysis technique to study the following types of reactions of free radicals formed by reaction of molecules with OH· free radical in aqueous solutions: a) organic free radicals + DNA, b) DNA· free radicals plus organic molecules, and c) DNA· free radicals plus organic free radicals. Such reactions are almost certain to occur in irradiated living cells. They may well constitute an important component of biological damage. Reaction b may be the route by which sulphydryl compounds, oxygen, and N-ethyl maleimide modify the effects of radiation on living cells. (cf Sub-Cellular Radiobiology Section, Dr. J. F. Ward). Depending on the organic molecule, one can study radiation induced reactions between DNA and proteins, DNA and amino acids, DNA and other small molecules found in living cells, DNA and protective compounds such as cysteine and cysteamine, etc.

Other projects: We hope to at least begin the following: a) solid state pulse radiolysis of single crystals of 5-bromouracil, b) determination of the thermal stability of free radicals in irradiated DNA and DNA constituents, c) study of the phenomenon responsible for a very short lived transient absorbance which has been observed in irradiated aqueous pyrimidine solutions, d) a more thorough investigation of electron reactions than carried out thus far, and e) extension of our *in vitro* systems to include nucleoprotein and viruses.

Exploratory Experiments with Cyclotron Produced Particles: In January of 1971 we expect a cyclotron to go into operation in the Laboratory. This cyclotron will produce 22 Mev protons, 11 Mev deuterons, and also energetic He^{3+} and He^{4+} ions. We plan to do exploratory experiments to determine effects of these particles on DNA and its constituents. A particularly interesting first experiment will be comparison of radicals produced in a single crystal by bombardment with protons with those produced by γ-rays.

16. EXPECTED RESULTS IN FY 1972

Research will be continued with the same general goals. Studies of radiation effects on nucleoproteins and related compounds will undoubtedly be continued, as will their gradual extension into more complex systems, including possibly viruses and bacteria. Specific problems undertaken will depend on the results of the preceding year's work; presently available information suggests that the following will be of importance: a) Studies of the modification of effects of OH· free radical on DNA. This will be done by pulse radiolysis of aqueous DNA solutions containing other components

such as various protective agents and cellular constituents; b) If preliminary experiments are encouraging, pulse radiolysis of single crystals of nucleic acid constituents; c) Increased emphasis on studies of electron and hydrogen atom reactions in both the solid state and solution. This work will include investigation of the oxygen effect; d) ESR studies of the structure of radicals formed in solution by $OH\cdot$, $H\cdot$, and e^-_{aq}. This project will require development of chemical or other new methods of producing the attacking species; e) If exploratory experiments with the cyclotron produced particles are promising, an extensive investigation of the action of these particles at the molecular level, and comparison with results obtained with γ-radiation.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Sub-Cellular Radiobiology

3. AEC Budget Activity No.:	4. Date Prepared:
06-01-02	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA

7. Person in Charge:	8. Project Term:
John F. Ward	From: 1964 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2	2	2
(b) Other Tech.	½	½	½
Total:	2 ½	2 ½	2 ½

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 30,600	$ 31,900	$ 33,000
(b) Materials & Services	6,500	7,400	8,400
(c) Indirect Expenses *	17,000	16,600	17,200
Total:	$ 54,100	$ 55,900	$ 58,600

* 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 1970

Ward, J. F., I. Johansen and J. Aasen: Radiosensitization by N-Ethyl Maleimide - A Model Chemical System, Int. J. Rad. Biol. 15, 163-170 (1969).

Ward, J. F., I. Johansen: A Mechanism for N-Ethyl Maleimide Radiosensitization, Biophysical Journal 9, A140 (1969).

Ward, J. F. and I. Kuo: The Effect of Chloride Ions on the γ-Radiation Induced Destruction of Nucleic Acid Bases in Aqueous Solution, Int. J. Rad. Biol. 15, 293-296 (1969).

Zimbrick, J. D., J. F. Ward and L. S. Myers, Jr.: Studies on the Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA. I. Pulse and Steady State Radiolysis of 5-Bromouracil and Thymine. Int. J. Rad. Biol. 16, 505-523 (1969).

Zimbrick, J. D., J. F. Ward and L. S. Myers, Jr.: Studies on the Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA II. Pulse and Steady State Radiolysis of Bromouracil-Substituted and Unsubstituted DNA. Int. J. Rad. Biol. 16, 525-534 (1969).

Ward, J. F. and I. Kuo: Aqueous Mononucleotides. A Model System for Investigation of the Mechanism of Single Strand Break Production in γ-Irradiated Nucleic Acids, Rad. Res. Society Abstract Ff 7, March (1970).

12. SCOPE OF THE PROJECT

The objectives of this project lie 1) in the elucidation of the mechanisms by which radiation damages cellular systems, and 2) in the investigation of chemical mechanisms which modify this damage.

Many different lines of evidence strongly suggest that radiation induced changes in DNA are the major cause of the biological effects of radiation (For a review, see Hutchinson, Cancer Res. 26, 2045-2052 (1966)). The weight of this evidence clearly justifies an extensive study of the mechanisms by which ionizing radiation alters DNA.

In our approach to the investigation of radiation damage in DNA we make use of model compounds and systems, as well as DNA itself, to measure the various types of damage produced.

In the second part of our studies we are attempting to define on a molecular level, the mechanisms of action of chemical treatments which are well known to affect the radiosensitivity of cellular systems.

These two lines of research form our main lines of investigation of radiobiological effects at a sub-cellular level. The summaries under Technical Progress in FY 1970 show how the problems are being approached and show how the radiation modifiers can act at a molecular level on a deoxynucleotide system - which is used as a model for DNA.

Our ultimate objective is the extrapolation of these findings to a cellular system - to be able to control to some extent the types of damage produced in DNA 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. and D. R. Howton 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; G. E. Adams and R. L. Willson, Mount Vernon, England; J. Hunt, University of Toronto, Canada; K. G. Zimmer, Institüt für Strahlenbiologie, Kernforschungzentrum, Karlsruhe, Germany; A. Müller, University of Regensburg, Germany; P. Howard-Flanders, Department of Radiology and F. Hutchinson, Department of Molecular Biology and Biophysics, Yale University, New Haven, Connecticut; I. Johansen, Norwegian Defense Research Establishment, Division for Toxicology, Kjeller, Norway; W. Garrison, Lawrence Radiation Laboratory, Berkeley, California.

Others include: National Institute of Health; University of California, Berkeley and Los Angeles; Stanford University; University of Notre Dame; Brookhaven National Laboratory; Oak Ridge National Laboratory; and several Laboratories in England, Russia, France, Australia, Germany, Israel and Japan.

14. TECHNICAL PROGRESS IN FY 1970

Model System for Investigation of the Mechanism of Production of Single Strand Breaks in DNA by Irradiation: Measurement of single strand breaks is currently the method of choice for assaying radiation damage to DNA irradiated *in vivo*. Investigations of the chemical nature of the single strand break and of the effect of radiation modifiers on break production are being carried out using a model system. Our model system for these investigations is dilute aqueous solutions of deoxynucleotides. Strand breaks occur as a consequence of damage produced in the sugar moiety of the DNA. Damage to the sugar of the deoxynucleotide results in the release of inorganic phosphate and is used as a measure of single strand breaks. In nitrogen or oxygen-saturated solution, inorganic phosphate is released with a G value of 0.4 (G value is the number of molecules released per 100 eV of energy absorbed).

However when the solution is saturated with nitrous oxide the yield of phosphate doubles. This suggests that OH free radicals are responsible for the sugar damage which leads to phosphate release (since nitrous oxide effectively doubles the OH radical yield by reacting with radiation produced hydrated electrons).

If a deoxynucleoside diphosphate is irradiated in solution the phosphate yield is approximately twice that of the monophosphate. Thus either 1) some sites of damage in the sugar molecule are not "scored" as phosphate release in the monophosphate or 2) the one damaged site leads to release of both phosphate groups from the diphosphate. Preliminary electrophoretic examination of the radiation products suggests that 2) is the mechanism involved.

Effect of Radiation Modifiers: A. Sulfhydryl Compounds: One of the mechanisms suggested for the radioprotective action of a sulfhydryl is a repair process where the sulfhydryl compound donates a hydrogen atom to a radiation damaged site in DNA. Since most radiation induced reactions of the purine and pyrimidine bases of DNA involve addition of radicals to double bonds, donation of a further hydrogen atom does not constitute re-

pair. However the hydroxyl radical reaction with the deoxyribose moiety of the DNA (discussed above) involves abstraction of a hydrogen atom - damage which can be repaired by reaction with sulfhydryl. Using the deoxynucleotide model system it was found that sulfhydryls (present in amounts insufficient to scavenge free radicals produced by water radiation) reduced the amount of inorganic phosphate released when the radiation is carried out in the presence of nitrogen. The reduction in phosphate yield was related to the sulfhydryl concentration in an expression involving the rate constant for the repair process. (Allowance was made for the various competitive reactions involved). Relative efficiencies of the repair reaction were measured for the sulfhydryl compounds cysteine, thiolactic acid and cysteamine and the ratios were 1, 1.2, 1.4 at pH 4. Variation of pH showed that the RSH form of the sulfhydryl compound was twice as efficient in carrying out the repair process as the RS^- form.

B. 5 Bromouracil: In a cooperative program reported by L. S. Myers, Jr., Chemical Radiobiology Section, a hypothesis for the molecular mechanism of radiosensitization by 5 bromouracil (5BU) was developed: In aqueous solution 5BU reacts with radiation produced hydrated electrons by a dissociative electron attachment process giving rise to a highly reactive uracilyl radical and a bromide ion. The uracilyl radical can abstract a hydrogen atom from a neighboring molecule to form uracil. Radiosensitization *in vivo* may be caused by the formation of uracil base in DNA and/or by increased single strand breaks produced when uracilyl radicals abstract hydrogen atoms from deoxyribose. The latter hypothesis was tested using the deoxynucleotide model for single strand break production. In order to observe reactions of the radicals produced from 5BU it was necessary to use solutions containing high 5BU concentrations and low deoxynucleotide concentrations; thus most of the free radicals produced in the water react with 5BU and any subsequent release of phosphate from the deoxynucleotide must be a result of reactions of radicals produced from 5BU. It was found that the organic radicals (formed from 5BU) increased the phosphate yield from deoxynucleotides suggesting that 5BU can sensitize by increasing the amount of single strand break formation. Parallel experiments using thymine, the base for which 5BU substitutes in DNA, showed no comparable yields of phosphate.

5BU also sensitizes cells to ultraviolet light; it has been suggested that the uracilyl radical, formed by homolytic cleavage of the C-Br bond, is involved here also. This was confirmed using α glycerophosphate as the source of phosphate. Again photolysis of α glycerophosphate in the presence of 5BU resulted in the release of phosphate whereas in the presence of thymine no phosphate was released.

C. N-ethyl maleimide: Reactions of the free radicals formed from water, by irradiation, with nucleic acids and their constituents produce organic free radicals which can react with N-ethyl maleimide (NEM). NEM binds to the organic free radical in the absence of oxygen. It was shown that NEM can compete with oxygen for the organic free radicals, and thus seems to act in an oxygen-like way. The binding of NEM radicals to DNA can occur at many sites in the molecule.

Hydrolysis of the DNA-^{14}C-NEM adduct by 6N HCl at 100°C for 2 hours results in the formation of several chromatographically resolvable compounds carrying the ^{14}C-NEM label. Similar hydrolyses of the four deoxynucleotide-^{14}C-NEM adducts were performed. Analysis of the chromatograms of these hydrolysates showed one major product common to the deoxynucleotides and DNA. This is probably formed by addition of NEM to a radical formed on

the deoxyribose moiety of these compounds.

In experiments run in the same way as the sulfhydryl experiments (section A above) it was found that the radiation yield of phosphate from deoxynucleotides is lowered by the presence of NEM during irradiation. This suggests that binding of NEM to the sugar radical does not give a product which releases inorganic phosphate. The presence of the NEM on the sugar moiety in vivo probably acts as an effective block to enzyme reactions on the DNA. This latter effect is the probable mode of radiosensitization of NEM.

15. EXPECTED RESULTS IN FY 1971

The above mentioned research will be continued along the lines suggested by the results obtained. For instance, attempts will be made to define the chemical changes which occur in the deoxyribose moiety of a deoxynucleotide which lead to the release of inorganic phosphate. The final chemical product after release of phosphate will have characteristic chemical properties for which an assay procedure can be devised. This assay procedure can then be modified to measure the extent of production of such compounds in DNA i.e. to measure single strand breaks. Using this assay the mechanisms of action of radiation modifiers on single strand break formation in DNA per se can then be investigated. In order to be sufficiently sensitive the assay procedures defined will be radiochemical in nature and will probably necessitate the use of dual labelling techniques.

Apart from continuation of the current research it is hoped to begin a project designed to investigate mechanisms of radiation induced mutation. Mutations probably arise from base alterations in the DNA of cells, which cause the code of the DNA to be misread. We would use the series of commercially available homopolydeoxynucleotides as a template for DNA or RNA polymerase. Alterations of bases produced by irradiation which lead to a read out of the wrong base can be determined: Incorporation of "wrong" bases into the newly synthesized polymer would be indicative of such a base change, this assay procedure could be made very sensitive by use of isotopic labels. Using this type of procedure it will be possible to measure the relative importance of this type of DNA damage compared to other DNA damage. Since major decisions regarding the use of nuclear energy in our society depend on a determination of the increase in the genetic load produced by low doses of radiation, these types of experiments will be useful in defining the relative significance of this type of radiation damage.

16. EXPECTED RESULTS IN FY 1972

Further work on phage or cellular systems with a view to examining the applicability of the molecular mechanisms already defined.

The mutation effects study mentioned above may be delayed until 1972 depending on support available. Further work on this system would involve using data obtained to devise a method of using a genetically well defined cellular system to investigate levels of radiation required to produce mutations.

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.:
06-01-02

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA

7. Person in Charge:
John E. Byfield, M.D.

8. Project Term:
From: 7/1970 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	0	0*	0*
(b) Other Tech.	0	0	0
Total:	0	0*	0*

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 0	$ 0*	$ 0*
(b) Materials & Services	0	500	3,000
(c) Indirect Expenses	0	0	0
Total:	$ 0	$ 500	$ 3,000

* Salary expense equivalent to two (2) Scientific Man Years being provided from other University sources at no cost to the Contract.

11. PUBLICATIONS DURING FY 1970

This project was not supported by the AEC. The following papers were prepared under other sponsorship:

Byfield, J. E.: Do the Products of mRNA Hydrolysis Cause 'Cloudy Swelling'? Experimentia 25, 1159-1161, 1969.

Byfield, J. E. and Y. C. Lee: The Effect of Synchronizing Temperature Shifts on the Synthesis and Translation of Replication-Supporting Messengers. Exptl. Cell Res.,(in press).

Byfield, J. E. and Y. C. Lee: Thermal Instability of Tetrahymena Ribosomes: Effects on Protein Synthesis. Biochem. Biophys. Res. Comm. 37, 806-812, 1969.

Byfield, J. E., and Y. C. Lee: Do Synchronizing Temperature Shifts Inhibit RNA Synthesis in Tetrahymena? J. Protozool., (in press).

Lee, Y. C., and J. E. Byfield: Properties of the In Vitro RNA Synthesizing Activity in Tetrahymena Macronuclei. Biochemistry (submitted).

Byfield, J. E., and Y. C. Lee: Effects of Synchronizing Temperature Shifts on the Translational Efficiency of Tetrahymena Messenger RNA. J. Protozool. (submitted).

Byfield, J. E., Y. C. Lee, and L. R. Bennett: Binding of Small Molecules to DNA Induced by Ionizing Radiation. Nature 225, 859, 1970.

Byfield, J. E., O. H. Scherbaum, and R. R. Lindstrom: The Induction of Synchronized Cell Division in Tetrahymena Pyriformis. Prog. Protozool. 3, 137, 1969 (Abstract).

12. SCOPE OF THE PROJECT

The overall goal of this investigation is to determine the acute effects of ionizing radiation on the expression of those gene sequences which control cytokinesis. Cytokinesis is used here to define the physical aspects of cell division (i.e., the production of two daughter cells from a single, mature, fully replicated cell). Past research has defined the mechanism by which the ciliated protozoan Tetrahymena pyriformis becomes synchronized following a series of cyclical temperature shifts. The results indicate that the synchronized Tetrahymena population appears almost ideal for an in depth study of the effects of ionizing radiation on the replication and expression of the DNA sequences coding for cell division. Beyond the obvious relevance to radiation biologists of the effects of radiation on cell division, it is noteworthy to point out that the expression of the gene sequences for cell division in Tetrahymena also offers an opportunity to study the effects of radiation on a complete, integrated entity of cellular differentiation. Unlike other cells, ciliates like Tetrahymena, begin the process of cell division by the organization of the second oral apparatus. The formation of this large structure follows a regular sequence in time and can be followed accurately by both silver staining and by electron microscopy. Our previous investigations have led to a considerable understanding of the biochemical events controlling this step in cellular differentiation; the additive benefit of having a controllable morphogenetic sequence to follow makes this cell system particularly useful. It is our object over the next two years to initiate an investigation of the effects of radiation

on each step in this complex developmental sequence. Eventually, we hope to explore the control of replication of the genes involved, their transcription and translation, and the ultimate formation of the quarternary structures they code for, including the oral apparatus and cleavage furrow.

13. RELATIONSHIP TO OTHER PROJECTS

Several other laboratories have been interested in the synchronized Tetrahymena system for several years. These include those at the Carlsberg Laboratory in Copenhagen (E. Zeuthen and colleagues): Drs. Holm and Christensen at the University of Lund, Sweden; G. Padilla, at Duke University; Dr. I. Cameron, University of Texas; Dr. J. Frankel, University of Iowa, and others. The group at Oak Ridge including Drs. Kimball, Whitson, and others have been interested in the effects of radiation on ciliates and have conducted a series of investigations. None of these has been directly concerned with the effects of radiation on synchronized Tetrahymena. Drs. Hanawalt and Brunk have used Tetrahymena (at Stanford) to demonstrate repair mechanisms in a eukaryote and have emphasized the close relationship between Tetrahymena and the higher eukaryotes. The number of laboratories utilizing Tetrahymena for biochemical studies has increased dramatically recently and will not be thoroughly catalogued here. As yet, however, there has been no extensive attempt to study the effects of ionizing radiation on the expression of the genes coding for cytokinesis such as is planned here.

14. TECHNICAL PROGRESS IN FY 1970

This program was not supported by the AEC. Work accomplished during the past three years under other sponsorship consists of 1) Effects of synchronizing temperatures on Tetrahymena ribosomes; 2) Nucleotide content of Tetrahymena ribosomal RNA; 3) The effects of high-temperature synchronizing shifts on RNA synthesis; 4) Hydrolysis of messenger RNA during synchronizing shifts; 5) Net loss in protein synthesis during synchronizing shifts-evidence for decreased messenger efficiency; 6) Cold-synchronization-similarities and disparities; 7) DNA replication during synchronization; 8) DNA replication and the Tetrahymena life cycle; 9) Reversibility of oral morphogenesis - the "division-protein" hypothesis; 10) Control of RNA synthesis in Tetrahymena; 11) Fractionation of Tetrahymena RNA; 12) In Vitro RNA metabolism in isolated Tetrahymena macronuclei; 13) $MnCl_2$-ammonium sulfate activated RNA polymerase; 14) Characterization of Tetrahymena nuclear-histones; 15) Rate of RNA synthesis and quantitative variation of histone and DNA in isolated nuclei; and, 16) Structural organization of the macronucleus and the sites of RNA.

15. EXPECTED RESULTS IN FY 1971

Our goals during the coming year of the investigation are limited. We plan to determine the effects of various doses of ionizing (Cobalt 60) radiation on the kinetics of synchronous cell division. In addition, we will determine the requirement for repair replication preceding cell division in those cells which are delayed in division by doses of irradiation which prevent half of the population from dividing. Finally, we will determine the morphologic effects of similar doses of irradiation (using silver-stained preparations) and will determine the effect of division-inhibiting doses on the stability of division-coding, messenger fractions.

16. EXPECTED RESULTS IN FY 1972

Based on previous results and on the data obtained under section 15, we will investigate the extent to which each step in the expression of division-coding genes is affected by division-inhibiting doses of Cobalt irradiation. Of particular interest are the following: a) How does damage to genes caused by ionizing radiation affect the transcription of messenger RNA in cells that contain two copies of each gene? b) Can ionizing radiation induce demonstrable damage to the mRNA coding for cell division and can this damage be defined by decay rates, variation in physical properties (such as MAK column chromatography), and in vitro coding properties? c) Does ionizing radiation produce sufficient damage to the translational machinery to produce faulty proteins? Can such faulty proteins be detected by variations in their electrophoretic properties or by the development of abnormal surface structures observable by silver staining or by electron microscopy? d) Is radiation-induced damage to cortical structures inherited in the same fashion as damage due to microsurgery or other manipulations? The latter has been well-documented by Sonneborn and his colleagues, Vance Tartar, and others. The possibility that radiation-induced damage to preformed cytoplasmic structures may be inherited may be particularly important in the long-term effects of radiation on post-reproductive 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:
Metabolic Radiobiology

3. AEC Budget Activity No.:
06-01-02

4. Date Prepared:
April - 1970

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: 1970

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	3/4	-	-
(b) Other Tech.	-	-	-
Total:	3/4	-	-

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 17,400	$ 0	$ 0
(b) Materials & Services	3,400	0	0
(c) Indirect Expenses *	8,500	0	0
Total:	$ 29,300	$ 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 IN FY 1970

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

de Vellis, J., and O. A. Schjeide: Effects of Ionizing Radiation on the Biochemical Differentiation of the Rat Brain in "Radiation Biology of the Fetal and Juvenile Mammal," M. R. Sikov and D. D. Mahlum (Ed.), pp. 857-875, U. S. Atomic Energy Commission, Oak Ridge (1969).

Schjeide, O. A., and J. de Vellis: Mechanisms of Radiation Damage in the Mammalian Fetus and Neonate. Ibid. pp. 919-942 (1969).

Schjeide, O. A., and J. de Vellis (Ed.): Cell Differentiation. Van Nostrand Reinhold Co., New York. 620 p. (1970).

de Vellis, J. and O. A. Schjeide: Effects of Ionizing Radiation on Nucleic Acids, Enzymes and Lipids During Postnatal Development of the Rat Brain. Ninth Annual Hanford Biology Symposium: Radiation Biology of the Fetal and Juvenile Mammal, Richland, Washington, May 5-8, 1969. (Abstract)

Schjeide, O. A. and J. de Vellis: Mechanisms of Radiation Damage in the Mammalian Fetus and Neonate. Ibid. (Abstract)

12. SCOPE OF THE PROJECT

The primary objective of the Metabolic Radiobiology Section, which has now been discontinued, was 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, studies were 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 included:

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 was 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.

Collaborative studies were carried out with: 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. Irvine; and Dr. Marshall R. Urist, U.C.L.A.

14. TECHNICAL PROGRESS IN FY 1970

Macromolecules in Oocyte Maturation

Evidence is presented that oocytes representing species from widely diverse phyla, including mammals, have several characteristic features in common. These include relatively large nuclear and cytoplasmic volumes, proximity to - and inter relationships with - "nurse" or follicular cells, microvilli and pinocytosis channels on their plasma membranes, stored or "masked" messenger RNAs in their cytoplasms, facility for sequestering macromolecules from the circulation which may play important informational roles in immediate and later development and tendencies toward a predominantly glycolytic metabolism as the egg advances in maturation.

Major emphasis in this research has been placed on macromolecules in the oocyte of the hen whose large size and exaggerated processes of maturation permits a relatively detailed and accurate examination of many of the above features.

Effect of Estrogens on RNA Metabolism in the Avian Liver.

Estrogens administered to a rooster will induce the synthesis of yolk proteins not previously present. These proteins (phosvitan, lipovitellin and lipoproteins) are present in the liver and blood of the laying hen. This project has investigated the changes in RNA metabolism which take place following administration of estrogens to a rooster. The new technique

of polyacrylamide gel electrophoresis has been adopted to isolate and characterize RNAs from the nucleus and cytoplasm of liver cells. Uridine-H^3 has been used to measure the synthesis and turnover of RNA fractions.

15. EXPECTED RESULTS IN FY 1971

Program discontinued due to resignation of Principal Investigator.

16. EXPECTED RESULTS IN FY 1972

Program discontinued.

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:
Biosynthetic Control

3. AEC Budget Activity No.:
06-01-02

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA

7. Person in Charge:
Armand J. Fulco

8. Project Term:
From: 1970 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	1	2	2
(b) Other Tech.	½	½	½
Total:	1 ½	2 ½	2 ½

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 20,100	$ 33,500	$ 34,600
(b) Materials & Services	2,200	2,700	2,800
(c) Indirect Expenses *	17,000	16,600	25,700
Total:	$ 39,300	$ 52,800	$ 63,100

* 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 1970

A. J. Fulco. Bacterial Biosynthesis of Polyunsaturated Fatty Acids. Biochim. Biophys. Acta 187, 169-171 (1969).

A. J. Fulco. The Biosynthesis of Unsaturated Fatty Acids by Bacilli II. Temperature Dependent Biosynthesis of Polyunsaturated Fatty Acids. J. Biol. Chem. (In press). June, 1970.

12. SCOPE OF THE PROJECT

Although the scope of the project has changed somewhat in response to new experimental results, the central aim remains the elucidation of the biosynthetic control mechanisms which regulate unsaturated fatty acid biosynthesis. As previously mentioned, unsaturated fatty acids are essential components in the membranes of higher animals and often, of plants and microorganisms as well. Unsaturated fatty acids of a defined chain length and double bond arrangement are specifically required to maintain normal membrane function in many organisms, and there are usually both quantitative and qualitative changes in membrane unsaturated fatty acid composition in response to changes in temperature and certain other environmental factors. In our work with bacteria (in particular, Bacilli) we are interested in three aspects of biosynthetic control.

1.) What are the factors which control the rate and type of unsaturated fatty acid biosynthesis in vivo.

2.) What are the biological mechanisms by which these factors influence unsaturated fatty acid biosynthesis - particularly the nature of the biological response to temperature.

3.) How do the unsaturated fatty acids, especially as components of complex lipids, function in cell membranes and in other possible metabolic and structural roles.

13. RELATIONSHIP TO OTHER PROJECTS

There is a strong relationship between this project and many of the projects undertaken by others in the laboratory, particularly the lipid studies of Dr. Mead's group and the studies of Dr. Howton and Dr. Nevenzel. Nationally, the work of Dr. Konrad Bloch (Harvard), Dr. P. R. Vagelos (Washington University, St. Louis) and C. F. Fox (University of Chicago) is strongly related to my work here. Ideas, information and experimental results are exchanged between these workers and myself, particularly at the Gordon Research Conferences on lipid metabolism each year.

14. TECHNICAL PROGRESS IN 1970

Bacillus licheniformis 9259 has been shown to synthesize two C_{16} polyunsaturated fatty acids from endogenous or added palmitate at 20° but not at 35°. Ready conversion of added palmitic acid labeled with C^{14} or H^3 to these fatty acids by cultures of B. licheniformis incubated at 20° permitted the isolation of sufficient amounts of material for the determination of structure and label distribution by physical and chemical methods. The major polyunsaturated component was shown to be 5,10-hexadecadienoic acid while a minor component had the 7,10-double bond structure. It was further shown that the biosynthesis of the major dienoic acid resulted from the cooperative action of two distinct desaturation systems. One system, which was

present at both 35° and 20° desaturated palmitic acid to 10-hexadecenoic acid. A second system, acting only at the lower temperature, resulted in the conversion of palmitic acid to 5-hexadecenoic acid. This system, which had previously been studied in B. megaterium, is apparently not present at 35° but is rapidly induced at 20°. Utilizing H^3-labeled 10-hexadecenoic and 5-hexadecenoic acids which were prepared biosynthetically, it was possible to show that, at 20°, either substrate could serve as a precursor for the biosynthesis of 5,10-hexadecadienoic acid by B. licheniformis. Indeed, they were both more effective in this regard than palmitic acid added in similar concentrations. The finding that B. licheniformis can carry out the conversion of palmitate to 5,10- and 7,10-hexadecadienoic acids apparently represents the first unequivocal demonstration of de novo polyunsaturated fatty acid biosynthesis in Bacteria.

Further experiments with the temperature-induced Δ^5-desaturating system of B. megaterium have shown that O_2, which is a cofactor for the desaturation reaction, is also necessary for the induction of the enzyme at 20°.

15. EXPECTED RESULTS IN FY 1971

Work will continue on attempts to prepare an active cell-free desaturating system from Bacilli. At present, a cell-free lysate from B. licheniformis shows some activity but it is not enough for in vitro studies. The mechanism of temperature induction will continue to be intensively studied, especially the relationship between O_2 and temperature-induced enzyme synthesis. The control events preceeding protein synthesis (i.e., messenger RNA biosynthesis) will be studied in relationship to temperature by utilizing selective inhibitors of RNA polymerase (i.e., rifampicin, actinomycin C). Work with a graduate student in Biological Chemistry (Norman Chang) will continue on the complex lipid metabolism of B. licheniformis.

16. EXPECTED RESULTS IN FY 1972

Assuming success in isolating, from Bacilli, cell free systems capable of Δ^5 and Δ^{10} desaturations, work will begin on the purification and characterization of the temperature-sensitive (Δ^5) and temperature-insensitive (Δ^{10}) systems. The in vivo studies of the control mechanisms involved in the temperature induction of the Δ^5 desaturating system will continue as will the studies concerning the roles played by the unsaturated fatty acids, as components of complex lipids, in membrane structure and function.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Physical Radiobiology

3. AEC Budget Activity No.:
06-01-02

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	2 ½	2 ½	2 ½
(b) Other Tech.	½	½	½
Total:	2 ¾	3	3

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 41,200	$ 44,000	$ 45,100
(b) Materials & Services	6,400	6,400	6,400
(c) Indirect Expenses *	25,400	33,200	34,300
Total:	$ 73,000	$ 83,600	$ 85,800

* 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 1970

Strickland, E. Hardin, Joseph Horwitz, and Carolyn Billups: Fine Structure in the Near-Ultraviolet Circular Dichroism and Absorption Spectra of Tryptophan Derivatives and Chymotrypsinogen A at 77°K. Biochemistry 8, 3205-3213 (1969).

Strickland, E. Hardin, Ernest Kay, and Leland M. Shannon: Effects of Denaturing Agents on the Phenylalanyl Circular Dichroism Bands of Horseradish Peroxidase Isoenzymes and Apoisoenzymes. J. Biol. Chem. 245, 1233-1238 (1970).

Horwitz, Joseph, E. Hardin Strickland, and Carolyn Billups: Analysis of the Vibrational Structure in the Near-Ultraviolet Circular Dichroism and Absorption Spectra of Tyrosine Derivatives and Ribonuclease-A at 77°K. J. Am. Chem. Soc. in press.

12. SCOPE OF THE PROJECT

The function of biological macromolecules depends critically on conformation as well as structure. Small changes in structure, such as those caused by ionizing radiation, may cause large changes in conformation, and significant changes in function as well. Some aspects of radiation damage may be mediated through alterations of moieties which control the conformation of macromolecules. Circular dichroism (CD) provides a means for assessing the conformation changes induced by ionizing radiation. Preliminary experiments suggest that low doses of radiation may alter the conformation of macromolecules, e.g., DNA and ribonuclease. A complete analysis of CD and absorption spectra will permit determining which parts of the molecule are involved in conformation changes. Detailed studies with constituents, however, are necessary before CD spectra can be interpreted with confidence. For this reason, our initial studies have focused upon the CD spectra of unirradiated amino acids and proteins. In addition, the effects of radiation upon each amino acid must be understood.

Special emphasis will be given the circular dichroism bands of proteins in the near ultraviolet. These conformation-dependent CD bands arise from the aromatic amino acid side chains and the disulfide bridges in proteins. Information achieved from the study of model compounds can be used to identify the CD bands of each type of aromatic side chain and also the disulfide bonds. In many cases, these near ultraviolet CD bands provide a sensitive probe for changes in the tertiary structure of enzymes, such as may occur in an irradiated protein.

The results of this work will provide new and sensitive criteria for assessing radiation effects in biological macromolecules and will give information on why the structural changes destroy function.

13. RELATIONSHIP TO OTHER PROJECTS

The effects of ionizing radiation on proteins 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.

Studies relating to the interpretation of circular dichroism spectra are being most actively carried out by Prof. John Schellman (Oregon), by Prof. Albert Moscowitz (Minnesota), by Prof. Oscar Weigang (Tulane), and by

Prof. Thomas Hooker (University of California at Santa Barbara), and also by Dr. Norman Simmons (LNMRB).

14. TECHNICAL PROGRESS IN FY 1970

Our studies of aromatic amino acid derivatives have yielded fundamental spectroscopic information which is essential to interpret the CD spectra of proteins. We have elucidated many factors that determine the shapes of the tyrosyl CD spectra. The vibronic transitions characteristic of monomeric tyrosine derivatives have been determined and analyzed. The distortions introduced by exciton CD effects have been observed in a dimeric tyrosine compound (L-tyrosyl-L-tyrosyl diketopiperazine). This information permitted a quantitative analysis of the near ultraviolet CD and absorption spectra of ribonuclease-A. The low temperature absorption spectrum revealed 3 exposed tyrosine residues and two types of buried tyrosine residues in ribonuclease-A. The CD contributions of each type of tyrosine and of the disulfide bonds were assessed. To our knowledge this is the first quantitative assignment of the near ultraviolet CD bands of any protein. Our techniques are applicable to proteins containing only a few tyrosine residues and lacking tryptophan.

The effects of denaturing agents on the phenylalanyl CD fine structure have been examined in the horseradish peroxidase isoenzymes. The intensities of the phenylalanyl CD bands are dependent upon the protein conformation. Surprisingly, in one case, their intensities increase after denaturation, which is contrary to the behavior of tyrosyl and tryptophanyl bands in these proteins. This behavior of phenylalanyl CD bands occurs, in part, because the observed CD spectrum is the average from over 20 residues. Our results emphasize the difficulty of interpreting CD spectra of proteins containing a large number of aromatic residues of the same type.

CD studies have continued on carboxypeptidase A. One important finding is that some of the tyrosyl and tyyptophanyl side chains have an unusually high degree of motion. This flexibility may be related to the catalytic mechanism.

Preliminary studies were begun to determine the effects of Co^{60} gamma irradiation on the CD and absorption spectra of ribonuclease. In the presence of either air or nitrogen during the irradiation, alterations of the exposed tyrosine residues seem to be extensive. (These studies are being carried out in collaboration with Dr. J. F. Ward).

15. EXPECTED RESULTS IN FY 1971

A study will be made of the effects of Co^{60} gamma irradiation on the conformation of enzymes by using circular dichroism. Radiation effects on both the peptide backbone and the orientations of aromatic amino acid residues will be examined. The effects of irradiation on the CD and absorption spectra of amino acid model compounds will be surveyed systematically. Attempts will be made to examine the optical absorption spectrum of the free radicals produced by irradiation of amino acids and proteins in the frozen state. (These studies are to be carried out in collaboration with Dr. J. F. Ward).

In order to obtain more precise resolution of overlapping aromatic bands in proteins, circular dichroism and absorption spectra will be recorded at $4^{o}K$.

The spectra of model compounds will be examined in non-polar solvents so that the characteristics of buried aromatic residues will be better understood. Special attention will be given to tryptophanyl spectra in non-polar solvents. The mechanisms giving rise to aromatic CD bands will be examined using diketopiperazines having a limited number of possible conformations.

16. EXPECTED RESULTS IN FY 1972

Studies of radiation effects on enzymes will continue. Circular dichroism will be used to examine possible allosteric transitions in enzymes showing feedback inhibition. Combined effects of radiation and feedback inhibitions will be considered.

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.:
06-01-02

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	2 ½	2 ½	2 ½
(b) Other Tech.	½	½	¼
Total:	3	3	2 ¾

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 32,800	$ 37,400	$ 38,500
(b) Materials & Services	8,000	10,400	11,500
(c) Indirect Expenses *	17,000	24,900	25,700
Total:	$ 57,800	$ 72,700	$ 75,700

* 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 IN FY 1970

de Vellis, J. and D. Inglish: Effect of Cortisol and Epinephrine on the Biochemical Differentiation of Cloned Glial Cells in Culture and of the Developing Rat Brain. Abstracts. pp. 151-152, 2nd Intern. Meet. Soc. Neurochem., Milan, Italy, Sept. 1969.

de Vellis, J., D. Inglish and F. Galey: Effect of Cortisol and Epinephrine on Glial Cells in Culture. In "Cellular Aspects of Growth and Differentiation in Nervous Tissue," Pease, Daniel (ed.), UCLA Forum in Medical Sciences, No. 14, University of California Press, Los Angeles, 1970.

de Vellis, J. and O. A. Schjeide: Effects of Ionizing Radiation on the Biochemical Differentiation of the Rat Brain in "Radiation Biology of the Fetal and Juvenile Mammal," M. R. Sikov and D. D. Mahlum (Ed.), pp. 857-875, U. S. Atomic Energy Commission, Oak Ridge (1969).

Schjeide, O.A. and J. de Vellis: Mechanisms of Radiation Damage in the Mammalian Fetus and Neonate. Ibid. pp. 919-942 (1969).

de Vellis, J., D. Inglish and R. Cole: Effect of Certain Hormones on Cloned Strains of Neurons and Glia in Culture. Abstract in "The Influence of Hormones on the Nervous System," Conference, June, 1970, New York.

Schjeide, O.A., F. Galey, E.A. Grellert, R. I.-San Lin, J. de Vellis and J. F. Mead: Macromolecules in Oocyte Maturation. Biology of Reproduction (in press).

Schjeide, O. A. and J. de Vellis (Ed.): Cell Differentiation. Van Nostrand Reinhold Co., New York. 620p. (1970).

a) de Vellis, J.: Enzyme Regulation During Cell Differentiation. Chapter 11, pp. 284-321.
b) de Vellis, J. and C. D. Clemente: Neural Cell Differentiation. Chapter 19, pp. 529-574.
c) Schjeide, O. A. and J. de Vellis: Introduction. Chapter 1, pp. 2-14.

de Vellis, J. and D. Inglish: Effects of Cortisol and Norepinephrine on Cultured Glial Cells - Function of Glial Cells. Abstract 3rd Winter Conference on Brain Research, Snowmass at Aspen, Jan. 25-31, 1970.

12. SCOPE OF THE PROJECT

The purpose of the Developmental Radiobiology section is to study the effect of ionizing radiation on the differentiation of brain cells in vivo and in cultures of cloned brain cells. Comparative studies also include such endogenous and exogenous factors as hormones and drugs. The aim of this work is to characterize the molecular, biochemical and ultrastructural effects of the above factors at the subcellular level, and to elucidate their mechanisms of action. Human and animal cloned strains of neurons and glial cells in culture are used and more cell lines will be established since cloned cell culture is the only technique which will allow us to (a) carry out quantitative biochemical studies on human material and (b) help circumvent the problem of cell heterogeneity and the lack of control of physical and chemical environment inherent to in vivo animal experiments. Brain cells are selected on the basis of their viability in culture and their ability to retain or express brain specific parameters in culture. Presently two brain specific effects by cortisol and norepinephrine have been obtained in a cloned cell

line of astrocytes. Such studies will help determine the function of glial cells (astrocytes and oligodendrocytes) and characterize further the molecular basis of neuronal functions as well as glial-neuronal interactions. The radiation effects on glial and neuronal differentiation can now be studied under controlled environment.

We are using in our studies cell lines cloned from differentiated neural tumors (i.e., retaining organotypic functions) since it is difficult to establish stable cell lines from normal tissues (which we are attempting to do). Our radiation studies on these model in vitro systems may be of importance in the design of treatments of human malignant tumors of the nervous system (see section, Radiobiology of Tumor Cells, J. de Vellis).

Ultrastructural and electrophysiological studies of neurons and glial cells in culture are conducted in collaboration with several investigators in other laboratories at UCLA.

13. RELATIONSHIP TO OTHER PROJECTS

This work is generally related to investigations in developmental biology under J. F. Mead, H. Herschman, I. Harary and L. Gerschenson in this laboratory. Other laboratories conducting related studies include: (a) Radiation Neurobiochemistry of the developing brain: V. Nair, University of Chicago; P. S. Timiras, University of California, Berkeley; D. Ford, S.U.N.Y., Brooklyn, New York; R. Brownson, University of California, Davis. (b) Biochemical and physiological studies of neurons and glial cells in culture: G. Sato, University of California, San Diego; M. Niremberg, N.I.H.; G. Tocco, Institute of Developmental Biology, Naples, Italy; P. Benda, Institut Pasteur, Paris, France.

14. TECHNICAL PROGRESS IN FY 1970

Effect of Ionizing Radiation on the Turnover of Brain Myelin, Lipids, Proteins and RNA in Subcellular Fractions: Our previous studies have shown that cephalic irradiation depresses markedly the synthesis of lipids, proteins, enzymes and RNA in the developing rat brain. Since the concentration of a compound depends on its rate of synthesis and its rate of degradation, the turnover rate of brain chemical constituents following irradiation was investigated in myelin, mitochondrial and microsomal fractions. Acetate ^{14}C was used as a marker. Animals were sacrificed at various intervals up to 3 months after injection. Myelin was found to turn over more rapidly in irradiated than in control animals. Analysis of individual lipids showed a differential effect. This increased catabolism added to the radiation induced inhibition of lipid synthesis explains why myelination is one of the most radiosensitive parameters in the postnatal brain. Preliminary studies indicate that there is also increased catabolism of RNA and proteins.

Effect of Ionizing Radiation on Cloned Glial Cells in Culture: Glial cells in culture derived from an astrocytoma showed greater radioresistance than the developing brain in vivo. When cells were grown with the radiosensitizer, bromodeoxyuridine, protein synthesis was reduced by 60% at a given dose. Induction of glycerolphosphate dehydrogenase by cortisol and lactate dehydrogenase by epinephrine was investigated in both normal and sensitized cells. These inductions are inhibited by ionizing radiation. The effects on unique RNAs was investigated by DNA-RNA hybridization techniques and gel electrophoresis. Radiation induced damage to DNA was studied.

Effect of Irradiation and Hormones on S-100 Protein Development in the Rat Brain: (see Developmental Biology, Dr. H. Herschman).

Hormonal Regulation of Differentiation in Neuronal and Glial Cells: The induction of glycerolphosphate dehydrogenase by cortisol and lactate dehydrogenase by norepinephrine in a cloned line of astrocytes has been further characterized. Substantial evidence has been obtained that cyclic AMP, an early mediator in the action of several hormones, is not involved in the enzyme induction. For epinephrine this appears to be an exception to the general rule which states that cyclic AMP mediates the physiological responses of epinephrine. There is evidence that transcription is the rate limiting step in the initial phase of induction while later translational control is rate limiting.

Electron microscopic studies have revealed that vacuolar electron dense material is depleted following treatment with cortisol. Nuclear changes were also observed.

The above effects were tested for cell specificity by using other cell cultures, Hela cells, lung cells, meningeal, ependymal and neuronal cells. These cells show none of the above observed effects.

Hormones tested on glial cell lines (rat and human) and neuronal cell lines (human and mouse) were the following: cortisol, corticosterone, estradiol, progesterone, norepinephrine, epinephrine, insulin, growth hormone, thyroid hormones, aldosterone, testosterone and others. Glucocorticoids and epinephrine affect glial cell differentiation. The experiments on neurons are still in progress.

The effect of irradiation and hormones on RNA species synthesis and degradation is investigated by gel electrophoresis and DNA-RNA hybridization techniques. This work is still in progress, much time was spent on developing techniques.

15. EXPECTED RESULTS IN FY 1971

Radiation induced degradation of DNA in the developing rat brain was previously reported. It is not known whether glial and/or neuronal cells are affected. Neuronal and glial nuclei will be separated by density gradient centrifugation and DNA content per nucleus determined. The effect of irradiation on non-chromosomal DNA (particularly in mitochondria) will be investigated because it is possible that some myelin (and other membrane) components are coded for in mitochondrial DNA. It is therefore of interest to know how radiosensitive mitochondrial DNA is compared to nuclear DNA. Besides chromosomes, DNA is found in the nucleolus, the centriole, mitochondria, many membrane systems, yolk granule and for plants, also in chloroplast. The physical state and transcriptional capacity of the DNA isolated from irradiated cells will be investigated by electron microscopy (shadow casting, and coupling techniques).

Regulation of protein synthesis and cell differentiation in mammalian cells is far from elucidated. Synthesis of a protein may be regulated at as many as 20 different sites at the level of transcription and translation (see publication #7). Our previous studies have pointed to a radiation lesion at the level of transcription during brain cell differentiation. Consequently detailed studies on DNA and RNA, which have been held back by lack of adequate precise methods for mammalian cells, will now progress

since methods have been developed and adapted in our laboratory, and current preliminary experiments seem satisfactory. Nucleic acid hybridization of unique genes in mammalian cells is now possible. The old method was not sensitive because it included the repeated part of the genome. A technique exists now to separate unique genes of the genome from the repeated part of the genome. Effect of irradiation and hormones on the expression of unique genes will be measured in the brain in vivo and in cultures of neurons and astrocytes.

Brain glycerolphosphate dehydrogenase and lactate dehydrogenase will be purified. This will allow us to measure the amount of enzyme-protein present in cells by using immunochemical methods and radiolabeling of proteins. The rate of turnover of induced enzymes and the rate of turnover of their mRNAs will be measured. Attempt will be made to elucidate the translational control(s) involved in the second phase of induction.

The effect of ionizing radiation on the growth of neuronal processes will be investigated in cell culture. It has been found by Dr. C. D. Clemente that cephalic irradiation of neonatal rats results in poor and disorganized growth of dendritic neuronal processes.

Electron microscopic studies and electrophysiology of cultured neurons will be carried out under various experimental conditions, i.e. irradiation, hormonal and drug treatments.

The change in the patterns of proteins synthesized following radiation or hormonal treatment will be investigated by double labeling with radioisotopes and separation of proteins by electrofocusing electrophoresis. This will be another means of measuring the number of genes turned on or off at the translation level and compared with the results obtained by DNA-RNA hybridization. This technique might also help elucidate translational control of protein synthesis.

16. EXPECTED RESULTS IN FY 1972

Radiation damage, repair and recovery will be investigated in several cloned cell lines of the nervous system. Recovery will be measured following split dose schedules. Unscheduled DNA synthesis will be studied in relation to the cell cycle and following irradiation. For this purpose cultured cells will be synchronized.

The greater radioresistance of some cell lines will be investigated at the chromosomal level by the use of cell hybrids obtained by two different techniques. Specific parameters measured will be induction of glycerolphosphate dehydrogenase and lactate dehydrogenase.

Attempt will be made to culture and study specialized neurons from various areas of the human embryonic brain.

The effect of ionizing radiation on the synthesis, distribution and metabolism of some neurotransmitters will be studied in cloned cultured neurons (human and mouse).

Electron microscope autoradiography technique will be adapted to study metabolism in cultured glial and neuronal cells. This will hopefully allow us to relate more easily structure and function in these 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:
Developmental Biology

3. AEC Budget Activity No.:	4. Date Prepared:
06-01-02	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA

7. Person in Charge:	8. Project Term:
Harvey Herschman	From: 1970 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{4}$
Total:	2 $\frac{1}{2}$	2 $\frac{1}{2}$	2 $\frac{1}{4}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 27,800	$ 32,400	$ 33,500
(b) Materials & Services	9,500	10,700	10,700
(c) Indirect Expenses *	17,000.	16,600	17,200
Total:	$ 54,300	$ 59,700	$ 61,400

* 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 1970

J. Lightbody, S. E. Pfeiffer, P. L. Kornblith, and H. R. Herschman. Biochemically Differentiated Clonal Human Glial Cells in Tissue Culture. J. Neurobiol. (In press).

J. E. Zuckerman, H. R. Herschman, and L. Levine. Appearance of a Brain Specific Antigen (The S-100 Protein) During Human Fetal Development. J. Neurochem. (In press).

L. Nonno, H. R. Herschman, and L. Levine. Serologic Comparisons of the Carbonic Anhydrases of Primate Erythrocytes. Biochim. Biophys. Acta. (In press).

S. E. Pfeiffer, H. R. Herschman, J. Lightbody, and L. Levine. Synthesis by a Clonal Line of Rat Glial Cells of a Protein Unique to the Nervous System. J. Cell. Physiol. (In press).

H. R. Herschman. S-100 Protein Accumulation in Developing Animals and Cell Cultures. UCLA Forum in Med. Sci. (In press).

C. W. Cotman, H. R. Herschman, D. Hansburgh. Subcellular Fractionation of Glial Cells. Amer. Soc. Neurochem., (1970). (Abstract).

12. SCOPE OF THE PROJECT

This project is oriented toward the study of organ specificity during development and differentiation, with special emphasis on the nervous system. We have been utilizing *in vivo* systems, observing the appearance of a brain specific protein in developing animals of a variety of species, and the effects of environmental manipulation on this phenomenon. We have also been utilizing clonal strains of cells isolated from functional tumors of the nervous system which produce this brain specific protein, to characterize the cellular events involved in its production.

Since joining the A.E.C. Laboratory in Sept., 1969, we have attempted to set up several other systems for the *in vitro* study of biochemical specificity in the nervous system. These include:

(1) Characterization of the Nerve Ending. An attempt to analyze the biochemically and immunologically unique aspects of the synapse. Nerve ending preparations have been made by techniques described in the literature and injected into rabbits to provoke an immune response. This antisera, when characterized, will be used to observe the degree of differentiation of clonal neuroblastoma (*vida infra*), analyze the developmental appearance of synaptic antigens, and compare species cross reactivity of this subcellular organelle.

(2) In Vitro Differentiation of Clonal Neurons. In the Laboratory I was previously associated with, my colleagues isolated a function neuron; a clonal cell line which in culture differentiates into a cell morphologically resembling a neuron and having the capacity to generate an action potential. The cells do not, however, make a functional synapse. We are utilizing biochemical and immunological techniques to ask whether portions of synapses are, however, made.

(3) Effects of the Peripheral Field on Neuronal Development In Vitro. We are culturing dispersed embryonic cockroach brain in the presence and absence of embryonic field tissue. The presence of embryonic tissue has been shown to promote the growth and differentiation of such preparations (Levi-Montalcini, personal communication). We propose a biochemical analysis of this classical embryological question in vitro.

(4) Genetic Effects of Innervation. In a series of experiments Bueller and his co-workers have shown that cross-innervation of fast and slow muscles of the cat results in the eventual alteration of physiological response in the muscle, i.e., slow becomes fast, and vice versa. These workers have recently shown changes in L.D.H. isozymes as a result of this cross-innervation. In order to show whether such an experimental procedure brings about concomitant changes in the myosins of these muscles(i.e., in the protein thought to be involved in the contractile process), we are preparing and characterizing antibody to the cat red and white muscle myosin, in collaboration with W. Mommaerts.

13. RELATIONSHIP TO OTHER PROJECTS

Our laboratory is interacting with a number of laboratories on this and other U.C. campuses. The study of muscle myosins is being done in collaboration with Dr. W. Mommaerts, Chairman, Dept. of Physiology, UCLA. The clonal neuroblastoma and embryo cockroach cultures are being characterized electrophysiologically with Dr. Dennis Hafemann, UCLA Dept. of Physiology. The study of the biochemical specificity of the nerve ending is being carried out with Dr. Carl Cotman, Dept. of Psychobiology, U.C. Irvine. Dr. Cotman prepared the nerve ending particles used for immunization. This collaboration has already produced what we consider to be a valuable analysis of the current methods of subcellular fractionation of nervous tissue (see last publication under 11). In addition, our interests bring us into close contact with Drs. Mead, Harary, Gerschenson, and de Vellis of this laboratory, due to our overlapping interest in neural development and cell culture.

14. TECHNICAL PROGRESS IN 1970

Since I have just joined the Laboratory, FY 1970 (or the part of it I have utilized) has been primarily a setting-up exercise. However, we have elicited antibodies to nerve endings and to the muscle myosins which we are presently characterizing with respect to specificity. In addition we have, I believe, made a very valuable contribution to the evaluation of currently used methods of subcellular fractionation of nerve tissue.

15. EXPECTED RESULTS IN FY 1971

In FY 1971 we hope to see a boom of information — the projects outlined above should become data-bearers. Thus we hope to be able to demonstrate unique macromolecules in nerve endings and to characterize these developments in vivo and perhaps in vitro. We also hope to have information on the nature of the intracellular events occurring in the density-dependent contact mediated induction of an organ-specific protein in vitro (see 4th publication under 11). Finally, we hope that we will have begun to get some biochemical answers on the effects of peripheral tissue on neuronal development, and its converse, the effects of neural innervation on genetic expression of peripheral targets.

16. EXPECTED RESULTS IN FY 1972

FY 1972 is a year and a half a way, and projection to our interests, let alone our results, is rather difficult. Undoubtedly the results of FY 1972 will depend on the progress of the preceeding year and the directions our research takes us during that time. It is safe to assume, however, that our interests will continue in the area of developmental neurobiology, the effects of one cell-type on another, and the interaction with the immediate environment.

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.:
06-02-04

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	4	3	3
(b) Other Tech.	1/4	1/4	1/4
Total:	4 1/4	3 1/4	3 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 57,200	$ 49,800	$ 49,800
(b) Materials & Services	5,500	5,600	5,600
(c) Indirect Expenses *	33,900	33,200	34,300
Total:	$ 96,600	$ 88,600	$ 89,700

* 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 1970

Heating Effects on the Thermoluminescence of Gamma Irradiated Soils, Soil Sci. 108: 1-10. 1969

Soil Thermoluminescence in Relation to Radiation Exposure Under Field Conditions. U. S. Atomic Energy Commission Report PNE-: In Press. 1970

Influence of Soil Heating on the Growth and Elemental Composition of Bean Plants. Soil Sci. 109: In press. 1970

12. SCOPE OF THE PROJECT

The objectives of the Soil Factors Section are to study the effect of ionizing radiation on soils and plants and the behavior of fission product and neutron-induced radionuclides in soils. Within the framework of these objectives, this Section is oriented primarily toward soil-plant interrelationship problems from a mechanistic point of view. It is believed that an understanding of the fundamental mechanisms is necessary in order to assess properly the biological cycling of radionuclides released into the environment whether by a nuclear detonation or reactor operation.

Currently, this Section is involved in:

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

(2) Thermoluminescence of gamma-and neutron-irradiated soils.

(a) Soil thermoluminescence as affected by temperature during exposure to gamma radiation.

(b) Thermoluminescence of soil fractions and minerals.

(3) Influence of heating and ionizing radiation on the availability on Sr90 and Cs137 and essential and nonessential elements to plants.

(4) Influence of soil organic matter on the plant uptake of Cs137

The scope and the objective of these projects are discussed below in the laboratory and controlled plant growth facilities. Some of the experiments on soil thermoluminescence will continue to be carried out by the use Cs137 radiation field in Rock Valley, Nevada Test Site. Field experiments in connection with the nuclear excavation studies under the Plowshare Program is anticipated.

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 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, Berkeley, California
University of Tennessee, Knoxville, California
Ecology Section, Oak Ridge National Laboratory, Oak Ridge, Tennessee

14. TECHNICAL PROGRESS IN FY 1970

Spurious thermoluminescence of six soils differing widely in physical and chemical characteristics was examined. As observed by previous investigators working with other materials, oxygen adsorbed on the surface of the luminescent particles appeared to be an important causative factor. The amount of "natural" spurious thermoluminescence depended on the kind of soil. Spurious (surface) glow and volume glow were increased considerably be exposing the soils to Co60 photons. The increase of the surface glow relative to that of the volume glow on irradiation depended also on the soil. One soil (Aiken clay loam) examined did not show detectable amounts of volume glow on radiation exposure up to 2,000 R, even though the spurious glow was present. Except for one soil (soil No. 4FF), the volume glow of the soils exposed to Co60 photons occurred predominantly in the low temperature range ($< 250^{\circ}C$), while the surface glow occurred predominantly in the high temperature range ($> 250^{\circ}C$). The large amount of volume glow induced in soil No. 4FF by irradiation appeared to be due to its high lime content.

In the study of the effect of soil organic matter on Cs137 uptake by plants, it was found that the uptake of Cs137 was reduced significantly by the addition of organic matter to the soil. In this experiment, soils with added organic matter were incubated in moist condition for various lengths of time up to 17 weeks, before cropping by a modified Neubauer Technique. Depending on the pre-crop incubation time, the percentage reduction of the average uptake ranged from 29 to 47, 52 to 59 and 34 to 75 for Hanford, Vina and Aiken soils, respectively.

An experiment was conducted to study the influence of clinoptilolite on Sr90 and Cs137 uptake by plants from soils contaminated only on the surface. Two forms of clinoptilolite (untreated and Ca-treated forms) were applied to the contaminated soil surface. The application of the untreated and Ca-treated clinoptilolite reduced appreciably the Sr90 contents of the plants. Depending on the treatment and the soil type, the percentage reductions ranged from 48 to 70, 54 to 77 and 44 to 77 per cent in the leaves, stems and fruits, respectively.

15. EXPECTED RESULTS IN FY 1971

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

The objective of this project is to determine the effect of surface application of clinoptilolite on the Cs137 and Sr90 uptake by plants grown in contaminated soils. The results obtained from one experiment has been summarized briefly above. At present, a long term experiment is in progress to determine the effect of clinoptilolite on the Sr90 and Cs137 release from soils as a function of time and

repeated cropping. This is a pot experiment using Ladino clover as test plant.

(2) Thermoluminescence of gamma-and neutron-irradiated soils.

(a) Soil thermoluminescence as affected by temperature during exposure to gamma radiation.

Under natural conditions, the soil temperature can vary from below freezing to around 70°C. Within this wide range of temperature, it is well known that soil temperature varies diurnally from day to day and season to season. The objective of this study is to determine the effect of temperature during gamma irradiation on the soil thermoluminescence. Several experiments on this project have been completed. Preliminary results indicate that temperature during irradiation period does have an effect. It is expected that this project will be completed this Fiscal year.

(b) Thermoluminescence of soil fractions and minerals.

The objective of this study is to determine the relative sensitivity of soil fractions and minerals to form induced thermoluminescence on irradiation.

(3) Influence of heating and ionizing radiation on the availability of Sr90 and Cs137 and essential and nonessential elements 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. At present, the experiments are in progress to determine the amount of water soluble and exchangeable cations and anions in different types of heated soils.

(4) Influence of soil organic matter on the plant uptake of Cs137.

The objective of this study is to determine the effect of soil organic matter on the uptake of Cs137 by plants. Data reduction phase of this experiment is in progress.

16. EXPECTED RESULTS IN FY 1972

Certain aspects of the projects in progress during FY 1971 will be continued. The initiation of the previously anticipated project to determine the effect of ionizing radiation in combination with heat on the reaction of several elements in soil will be deferred to this FY. It is possible that this Section may be involved also in a still undetermined project under IBP (International Biological Program).

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.:
06-02-04

4. Date Prepared:
April - 1970

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 Romney

8. Project Term:
From: 1953 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	4	3	3
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total:	4 $\frac{1}{4}$	3 $\frac{1}{4}$	3 $\frac{1}{4}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 62,900	$ 49,800	$ 50,900
(b) Materials & Services	6,200	7,400	7,400
(c) Indirect Expenses *	42,400	33,200	34,300
Total:	$ 111,500	$ 90,400	$ 92,600

* 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 1970

Effect of Gamma Ray Irradiation in Stability of FeEDDHA Solution at Two pH Levels. Agronomy Jour. 61:652-653, 1969 (with A. Wallace, P. Wieland, M. Leo and G. A. Wallace).

Effect of Soil Temperature and Zinc Application on Yields and Micronutrient Contents of Four Crop Species. Agron. Jour. 61:567-568, 1969 (with A. Wallace, V. Q. Hale and R. M. Hoover).

Radioactive Fluorine 18 in Soils and Plants. Soil Science 108:419-423, 1969 (with R. A. Wood and P. Wieland).

Effect of Phosphorus Level on Micronutrient Content of Franseria dumosa Phyton 26:(In Press)(with A. Wallace and R. T. Muller).

Zinc Sources on Micronutrient Contents of Golden Cross Bantam Corn. Soil Sci.(In Press) (with A. Wallace).

Persistence of Plutonium in Soil, Plants and Small Mammals. Health Physics (Accepted for Publication) (with H. M. Mork and K. H. Larson).

12. SCOPE OF THE PROJECT

Research objectives of the Plant Factors Section are three-fold in scope: (1) improve understanding of the function of plants in the biological cycling of radioactive isotopes contaminating the environment, (2) continue to evaluate the irradiation effects on vegetation from fallout contamination originating from Plowshare nuclear testing events at the Nevada Test Site, (3) investigate the mineral nutritional characteristics of desert vegetation and the subsequent response of desert plants to the manipulation of environmental factors known to influence plant growth and development. These objectives aim to a better understanding of the effects of radioactive fallout damage to vegetation, of ecological relationships among desert plants and soils, and also of the fundamental mechanisms involved in soil to plant transfer of radionuclides and mineral elements in the desert ecosystem.

Investigations are conducted in both field and controlled laboratory environments in order to help bridge the gap between practical and theoretical problems arising from radioactive fallout contamination, and also to increase understanding of the functional and homeostatic factors involved in maintaining the integrity of vegetation in the desert ecosystem. Results should help establish parameters for assessing the consequences to man (and other living components of the environment) resulting from radioactive contamination of the environment, and also increase knowledge of certain mineral nutritional characteristics and physiological peculiarities of desert plants. Much of the information derived from this work should be directly applicable to the interests and needs of the U.S./I.B.P. Desert Biome Program (see schedule 189 on IBP Program).

13. RELATION TO OTHER PROJECTS

Research activities are correlated with other investigators in the Environmental Radiation Division, viz., A. Wallace, O. R. Lunt, N. Q. Hale, H. Nishita, F. B. Turner and R. A. Wood. Some related research is conducted at the Biology Department Battelle-Northwest Laboratory; Radiation Ecology Section, Oak Ridge National Laboratory; Biology Department,

Emory University; Biology Division, Lawrence Radiation Laboratory; E. G. and G. Santa Barbara; Desert Biome Program, Utah State University.

14. TECHNICAL PROGRESS DURING FY 1970

Work continued in the fallout areas of three Plowshare excavation tests at the Nevada Test Site. Additional documentation was made of the recovery and succession of vegetation in areas damaged by fallout from Palanquin and Cabriolet. Mixed grasses, viz., Sitanion hystrix, Oryzopsis hymenoides, and Poa nevadensis, account for much of the vegetative cover in the areas destroyed by fallout four years after Palanquin and two years after the Cabriolet event. The original vegetation in these areas was predominantly Artemisia tridentata and A. arbuscula. In some areas of the Palanquin pattern new Artemisia seedlings have become established (3-4 years old) at densities varying from 10 to 20 percent of the original shrub population. We find evidence of much higher density of one year old Artemisia seedlings having germinated in the Cabriolet patterns, but insufficient time has lapsed post-shot to determine their degree of survival. Along an arc in the projected fallout pattern at 5000-6000 ft from ground zero of Schooner, we covered plots of native vegetation with sheets of polyethylene plastic for a two week period to protect vegetation from fallout debris. Observations during the first year post shot showed the same sequence of radiation damage as was observed at Palanquin and Cabriolet. Vegetation that was subsequently killed began to show a yellowing of foliage about four months post shot which intensified until defoliation and death occurred. An index of irradiation damage on Artemisia species in partially killed areas is the failure of inflorescence to develop during the early spring months. In Juniperus osterosperma, the failure to develop its small blue-grey fruit is an index of radiation damage. Considerable asymmetry in shrub damage occurs with these two plant species; it is common to see infloresences or fruits developed only on the side of the shrub facing away from ground zero, or on parts of the plant shielded from fallout debris. In the areas where total or partial kill occurred from Schooner fallout, the vegetation protected by polyethylene plastic covers survived without damage. This has given further assurance that damage to vegetation from fallout primarily is caused by beta irradiation from fallout debris deposited on plant foliage.

Work continued at our environmental study plots near Mercury, Nevada, where we are investigating the effects of irrigation, nitrogen and phosphorus fertilization, herbicides and insect control on primary productivity. A census of shrub species in 25 plots was completed and a computer analysis of the plant community structure is now underway to provide understanding of relationships among individual plants, species population levels, and community levels for shrubs indigenous to this area. Samples were obtained in areas adjacent to these study plots to provide data on biomass to shrub volume relationships which we shall use in our computer analyses, and which also may be useful to the Desert Biome Program of the U.S./I.B.P. Supplementary irrigation equivalent to 9 and 12 inches of rainfall above that which naturally occurred during the past two years has caused some interesting changes. Plots 100 ft in diameter in this area normally have from 0 to 10 Salsola plants, while irrigated plots had from 1,000 to 1,400 Salsola plants in 1969. Clumps of mixed grasses responded to additional moisture as did Lycium andersonii, Franseria dumosa, Grayia spinosa, Eurotia lanata and Sphaeralcea parvifolia. These shrub species not only produced more new biomass but they also retained their foliage for several weeks longer than did the same species growing in adjacent

plots without supplementary irrigation. Subsequently, there occurred such a problem during the late summer and fall months from jackrabbits eating the succulent foliage that it was necessary to fence the study area. Measurements of primary productivity were made in the environmental study area at Mercury and in the radiation study area in Rock Valley where some indications of gamma radiation damage to Ephedra nevadensis have appeared.

We have developed a battery operated, ten channel recorder capable of continuously or intermittantly monitoring soil and air temperature from ten different profile sensing stations consisting of the Fenwal KA31L4 thermistor. A network of soil temperature monitoring sites has been established at most of our environmental study plots and at other locations of special ecological interest in the transitional zone between the Mojave and Great Basin deserts generally located in the southern part of the Nevada Test Site. Among other environmental factors measured continuously or intermittantly were wind speed and direction, maximum and minimum air temperature, rainfall, and soil moisture at 3-6 and 9-12 inch depths.

A soil survey was started and the soil profiles at 75 sites have been characterized. Samples of the soil profiles and the major shrub species indigenous to each sampling site were collected. Soil samples are being processed for chemical and physical properties, and the plant samples are being analyzed for mineral element content. Our capability for analyzing plant materials has been greatly enhanced by the computerized emission spectrometric procedures recently developed with G. V. Alexander (Spectroscopy Section). A comprehensive survey of some 20 elements in desert plant species is in progress and over 6,000 samples have been analyzed to date. These data are stored in form for direct access by computer in order to fulfill the needs of our continuing research program and be available to the U.S./I.B.P. Desert Biome Study.

Preliminary work was started on the Sturtevant project consisting of study site locations and the collection of background soil and plant samples. Laboratory work is in progress to investigate the gamma irradiation sensitivity of some plant species indigenous to the Sturtevant test area. Some laboratory studies of the mineral nutritional and physiological characteristics of several desert plant species are in progress.

15. EXPECTED RESULTS IN FY 1971

We shall continue to investigate biological cycling of radionuclides and the effects of environmental radiation on vegetation in areas contaminated with fallout from Plowshare Program events, with emphasis being placed on the Sturtevant project should it be permitted to occur. In any event laboratory studies of the effects of gamma radiation on desert vegetation will be continued in order to determine the sensitivity of the prominant desert plant species to this kind of radiation. Work in our environmental study plots will continue in order to evaluate the effects of certain climatic and edaphic factors on primary productivity, and emphasis will continue on the computer analysis of the plant community structure in these environmental study plots. An intensive mineral analysis of plant samples will be continued in conjunction with the soil survey of major study areas at the Nevada Test Site. Work will begin on the plant studies relative to the U.S./I.B.P. Desert Biome validation study site in Rock Valley (see schedule 189 for IBP Program). Initial emphasis will be placed on characterizing the vegetation and setting up a program for environmental measurements in the validation study site.

Laboratory investigations will be continued at UCLA to support and confirm findings from field studies with emphasis being placed on further evaluation of the mineral nutritional requirements and physiological characteristics of desert plants. Many of these research activities will continue to be carried out in collaboration with A. Wallace and V. Q. Hale of this Division. Results from completed phases of research will be submitted for publication.

16. EXPECTED RESULTS IN FY 1972

We expect to continue all phases of field and laboratory work in progress. Continued involvement with Plowshare Program events is desirable to the extent of helping reach our research program objectives. We hope to have firmly established the IBP Desert Biome Program at Rock Valley with plant studies in progress in the validation study site and in nearby process study sites. Computer analysis of the plant community structure of our environmental study sites will be continued and extended to other sites as needed. We hope to complete the soil survey of major study areas of Nevada Test Site and prepare a monograph on the mineral relationships of desert plants and soils. The second census of the Mercury study plots should be completed to further evaluate the response of desert vegetation to manipulation of environmental factors limiting primary productivity. We expect to have made considerable progress in further understanding the effects of radiation on desert vegetation and also the mineral nutritional requirements and physiological characteristics of most of prominent shrubs in this 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.:
06-02-04

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Howard Hawthorne

8. Project Term:
From: 1963 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	3	2 1/2	2
(b) Other Tech.	1/4	1/4	1/4
Total:	3 1/4	2 3/4	2 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 45,900	$ 37,700	$ 31,100
(b) Materials & Services	11,500	5,800	5,800
(c) Indirect Expenses *	25,400	24,900	17,200
Total:	$ 82,800	$ 68,400	$ 54,100

* 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 1970

H. A. Hawthorne. Effect of ammonium nitrate on uptake of Sr90 from soils by tomato plants. Soil Science (Submitted).

H. A. Hawthorne and K. H. Lu. Elimination of inefficient parameters from models of grisystem with path analysis. Health Physics (Submitted).

H. A. Hawthorne, K. H. Lu and S. D. Zellmer. Direct and joint contributions by plant organs to variance in plant radiostrontium content. Radiation Botany (Submitted).

12. SCOPE OF THE PROJECT

A forty-acre, self-sufficient dairy farm was rented as the sampling domain for studies in the fallout microcosm. Samples of specimens of twenty-six components of the system were collected to establish fission product baselines, for measurement of fallout input, and definition of radionculide sinks. Collection of samples of specimens was carried out three and one-half years and all collections at the site were terminated in 1968, as was specimen processing for radiochemical analyses. Data from the samples is to be used for validation of mathematical models and development of comprehensive simulation.

13. RELATIONSHIP TO OTHER PROJECTS

Collaborative studies have been carried out at Nevada Test Site with Ecology of the Nevada Test Site (480342) since 1963; cooperative experiments have traversed areas of mutual interest with Soil Factors (480310). Large groups at Davis, Cal. (Institute of Ecology) and at Oak Ridge National Laboratory (Radiation Ecology Section) have undertaken analytical and simulation studies that in principle and methodology are comparable to this study.

14. TECHNICAL PROGRESS IN FY 1970

The specific accomplishments are indicated by the titles of item 11. In general, two main activities were pursued. Denial of access to computing facilities from 1966 through mid-1968 halted data reduction for specimens that were analyzed for radionuclide content. Computing programs made in 1964-1965 became obsolete and were not revised. On recommendation of UCLA consultants in August 1968, the data reduction programs were to be rewritten. This has been in progress for nearly two years, and should be finished by FY 1971. Analyses of milk, feeds, and feces for stable strontium, cesium, potassium, calcium, rubidium, magnesium and phosphorus proceeded as equipment has come.

15. EXPECTED RESULTS IN FY 1971

Data reduction on the computer should conclude. Publication of results will proceed as the data bank is assembled and interpretations, including computerized statistical evaluation, are constructed for the agrisystem.

16. EXPECTED RESULTS IN FY 1972

Isothermal investigations of soil moisture diffusion, induced by non-thermal energy input and resulting in measurable entropy changes, will start.

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.:	4. Date Prepared:
06-02-04	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA and Nevada Test Site

7. Person in Charge:	8. Project Term:
Arthur Wallace	From: 1958 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2 1/2	2 1/4	2 1/2
(b) Other Tech.	1/4	1/4	1/4
Total:	2 3/4	2 1/2	2 3/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 67,600	$ 41,000	$ 48,700
(b) Materials & Services	5,400	7,400	7,400
(c) Indirect Expenses *	42,400	24,900	25,700
Total:	$ 115,400	$ 73,300	$ 81,800

* 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 1970

Wallace, A. and E. F. Frolich. 1969. Vegetative propagation of woody shrub species from the northern Mojave and southern Great Basin deserts. Internatl conf. on Arid Soils in a Changing World, Tucson, Arizona, June 3-13, 1969. Abstract.

Resnik, M. E., O. R. Lunt, and A. Wallace. 1969. Cs, K, Sr, and Ca transport in two different plant species. Soil Sci. 108 (1):64-73.

Wallace, A., E. M. Romney, V. Q. Hale, and R. M. Hoover. 1969. Effect of soil temperature and zinc application on yields and micronutrient content of four crop species grown together in a glasshouse. Agron. Jour. 61:567-568.

Romney, E. M., A. Wallace, P. A. T. Wieland, M. W. M. Leo, and G. A. Wallace 1969. Effect of gamma ray irradiation on stability of FeEDDHA solution at two pH levels. Agron. Jour. 61:652-653

Wallace, A. and R. T. Mueller. 1969. Effect of chelating agents on the availability of plants of carrier-free FE^{59} and Zn^{65} added to soils to simulate contamination from fallout. Soil Sci. 33(6):912-914.

Hale, V. Q. and A. Wallace. Effects of chelates on uptake of some heavy metal radionuclides from soil by bush beans. Soil Science (In press)

Joven, Claricia B., V. Q. Hale and A. Wallace. DTPA pH ^{59}Fe, ^{65}Zn, ^{137}Cs, ^{241}Am, ^{210}PB by orange leaves. Proc. Amer. Soc. Hort. Sci. (In press)

Wallace, A. Water use in a glasshouse by Salsola Kali grown at different soil temperatures and at limiting soil moisture. Soil Sci. (In press)

Wallace, A., E. M. Romney and R. T. Mueller. Effect of the phosphorus level on the micronutrient content of Franseria dumosa. Phyton (In press)

Kaaz, H. et. al,. Differential effect of a chronic dose of gamma irradiation on shrubs in Northern Mojave Desert. (submitted)

Wallace, A., V. Q. Hale, G. E. Kleinkopf, and R. C. Huffaker. Carboxydismutase and phosphoenolpyruvate carboxylase activities from leaves of some plant species from the Northern Mojave and Southern Great Basin deserts. (Submitted)

12. SCOPE OF THE PROJECT

Two major goals in this study are to develop information that contributes to an ecosystem approach to the problem of ionizing irradiation effects on biological systems and to develop information or specific aspects of irradiation on desert plants and of cycling of radionuclides in desert systems. To achieve these goals it is necessary to study many aspects of the ecology and physiology of undisturbed systems. These studies and those of the mission go hand in hand.

To achieve these two goals there is considerable cooperation with other investigators. The most important team project is that of the ^{137}Cesium

source in Rock Valley where an area has been irradiated since 1964 with a chronic level of the irradiation. In this area we are as a group moving more and more toward an ecosystem approach in this particular study. It appears that there may be some collaborative effort in this particular area with the International Biological Program (IBP) and this effort if it materializes will be closely correlated with the irradiation studies to achieve the ecosystem study needed by the U. S. Atomic Energy Commission. A model of the ecosystem should become available through cooperation with the desert biome study of the IBP. This model can be used for predicting and testing what some of the subtle effects of the irradiation on the ecosystem can be. Major effects of the irradiation, of course, are continuously being studied and observed. This opportunity to collaborate with the IBP can make it possible to achieve one of our most important goals. The role of this section in the studies in Rock Valley involves plant assessment and inventories. Future studies will involve phenology, age-structure, primary productivity including root and stem growth, seed production. Special attention will be given to the irradiated area in which last year one of the major perennial species, Ephedra nevadensis, was greatly affected by the five years of irradiation. No other effects on plants have been observed to date.

The second important team project are studies relating to Plowshare events. We are organized to meet some of the needs of the Atomic Energy Commission in assessing some of the close-in effects of Plowshare testing. The role of this section in cooperation with other groups in this type of study is assessment of irradiation effects on plants and radionuclide uptake by plants and cycling in the system.

Other efforts of this section relate to ecological studies of control and manipulated environments to determine limiting factors in desert ecosystems and some aspects of mineral and nitrogen cycling. Special emphasis, again in a team basis with other investigators, is given to an evaluation of the structure of the perennial component of the biota in desert ecosystems. This type of information is needed for ecosystem studies.

13. RELATIONSHIP TO OTHER PROJECTS

Research is correlated 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); Radioecology 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 (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, Michigan AT (11-1) 888; Department of Horticultural Science, University, of California, Davis, California 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, California; Southwest Radiological Health Lab., Las Vegas, Nevada - B.Mason;

Utah State University, Environmental Sciences Department - D. Goodall. 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 1970

In cooperation with Dr. E. M. Romney (480311) a field - plant study was continued from FY 1969. This was designed to further assess limiting factors in the primary production of the particular ecosystem and to determine information on plant community structure. This background information is highly essential to studies relating to effects of ionizing irradiation on a desert ecosystem. Plant assessments in Rock Valley indicated that only one shrub species had been measurably affected by the 5 years of irradiation. The effect correlates with nuclear volume information. Additional information was obtained on revegetation associated with Plowshare events at the Nevada Test Site. Several aspects of basic plant and soil ecology were continued. Limiting and regulating factors on plant productively including moisture, nutrients, and soil temperatures were evaluated. A large number of plant samples were assayed for nutrient contents. Plans were made for more detailed ecosystem and nuclide cycling studies.

15. EXPECTED RESULTS IN FY 1971

Inventories and assessments of plants will be made in the Rock Valley area in relationship to the irradiation source and to the projected needs of the International Biological Program towards a model representing the desert biome. Emphasis will be on population, age structure and productivity. We believe that such a model really is necessary before we can determine what ionizing irradiation actually does to an ecosystem. Previous studies will also be continued with some emphasis on Plowshare activities. Considerable previous data will be published.

16. EXPECTED RESULTS IN FY 1972

Projects will be continued through this year and data will be assembled into manuscript and made available for other people.

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.:	4. Date Prepared:
06-02-04	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA and Nevada Test Site

7. Person in Charge:	8. Project Term:
B. Maza (Acting)	From: 1959 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2 1/2	2 1/2	3
(b) Other Tech.	1/4	1/4	1/4
Total:	2 3/4	2 3/4	3 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 47,500	$ 39,900	$ 47,600
(b) Materials & Services	5,500	5,800	6,900
(c) Indirect Expenses *	25,400	24,900	25,700
Total:	$ 78,400	$ 70,600	$ 80,200

* 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 1970

French, N. R., B. G. Maza and H. W. Kaaz. 1969. Mortality Rates in Irradiated Rodent Populations. In: Symposium of Radioecology, Nelson, D. J., and F. C. Evans (editors). U. S. Atomic Energy Commission (Biology and Medicine) Report. CONF-670503 (TID04500).

French, N. R. 1969. Radiation Sensitivity of Rodent Species. Nature, vol. 222, No. 5197, pp. 1003-1004

French, N. R. 1969. Effects of Irradiation of a Desert Ecosystem for Five Years. In: Proceedings of the Radioecology Symposium, Cadarache, France, (In press).

French, N. R. 1969. Simulation of Dispersal in Desert Rodents. In: Proceedings of the International Symposium on Statistical Ecology. Patil, G. P., E. C. Pielou and W. E. Waters (editors). (In press)

12. SCOPE OF THE PROJECT

Studies have been in progress for several years in the Rock Valley area where a 33,600 curie ^{137}Cs source is located. Dr. N. R. French has been in charge of the program but has left the organization and plans are under way to replace him this fiscal year. This project was designed to learn effects of the radiation field not only on species of mammals but also to learn of possible indirect effects on the total ecosystem. The project continues with these as goals. Important effects of the irradiation on some of the mammals have previously been reported and indicate that fertility has been decreased by the levels used. Other effects to be studied will be specifically known when the new investigatior is on the job but in the meantime the staff in this section is busy completing certain aspects of the previous programs. Examples of these are as follows:

1. Analysis of survival in Rock Valley rodent population. This is completed for the main species, P. formosus, but needs to have results from other three species integrated for total picture of survival.

2. Analysis of fertility in Rock Valley rodent population. Preliminary analysis of fertility in the main species is complete, needs only to be critically reviewed. It is unlikely that data are sufficient for analysis of other species, but this must be considered.

3. Food consumption by Rock Valley rodents. The objective is to make precise qualitative determination of the foods utilized by the rodents at all times of the year, and to make quantitative estimates of the utilization of major food types. Completion of this portion of the work depends on Arnold Aschwanden's efforts at Mercury.

4. Food production in Rock Valley study area. The bulk of the quantitative determination of production of rodent food in Rock Valley has been accomplished by counts and sample harvests of the annual and perennial plant seed production during three successive years in Rock Valley and the laboratory evaluation

of seeds in the samples by Herb Hill. Production of fruits and vegetative parts that are known to be utilized by the rodents was also included. A fair amount of laboratory counting remains to be done.

5. Correlation analysis. The important information to be derived from these studies is the evaluation of interaction between different processes of the rodent population and between processes and environmental factors. These include:

   a. food production and food utilization
   b. rainfall and food production
   c. rainfall and rodent production
   d. temperature and food production
   e. temperature and rodent production

6. Home range analysis. A fundamental problem of ecology is being examined by utilizing the extensive data accumulated in the Rock Valley rodent study.

7. Development of external reproductive characters. Criteria for characterization of the reproductive condition have been developed by field examination of rodents for several years. Adequate description and illustration of these characters will be of value to most field workers.

8. Energy requirements of desert rodents. Integration of the results of individual seasonal energy requirements by rodents, utilizing the method of double labeled body water, with the accumulated information on population density will provide information on interaction between the mammalian populations and environmental resources that have never before been available.

9. Age estimation of rodents by tooth wear. A modification of the technique that has been long utilized for game species permitted age estimates of rodents collected for laboratory examination. Development of a technique useful for unrooted or ever-growing teeth would be a valuable contribution, but requires much more experimental work.

A. Plans for continuation of field sampling include the following:

1. Determine peak population density in post-reproductive period. This will require eight weeks of trapping in the spring by our standard method (three successive nights of trapping in each grid), covering each grid twice (one month apart) in order to hit the population at its peak. Knowledge of the peak population density will indicate long-term changes in reproductive requirements of the population from the environment in terms of energy and space.

2. Determine population density in the fall of the year. This will require a similar effort. Results will indicate survival of young born in the spring of the same year, and will provide a base-line for estimation of over-winter survival.

3. Determine pre-breeding population density. Requires a repeti-

tion of the previous sampling (making a total of 6 months full-time field work for two technicians). Results will indicate over-winter mortality and provide a base-line for estimation of population productivity.

4. Natality and pre- and post-natal mortality. A careful evaluation of female rodents trapped and sacrificed in lower Rock Valley, including histological examination for corpora lutea of pregnancy and placental scars, and counts of embryos will be done. Examination of specimens would have to be more precise than in the past and sample size must be larger. Age estimates of females should also be made. Results would permit estimation of potential productivity of the populations that are studied by live-trapping, and possibly an evaluation of radiation effects on the population.

13. RELATIONSHIPS TO OTHER PROJECTS

In the Rock Valley study area, where these field investigations have been conducted during the last nine years, other investigators of this laboratory have been 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 of 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 1970

Several of the items listed above will be completed during the year. These include: a) Analysis of survival in Rock Valley rodent population. b) Food consumption by Rock Valley rodents. c) Food production in Rock Valley study area. d) Home range analysis.

A disseration by a graduate student entilled The Metabolism of Four Species of Desert Rodents in their Natural Environments is being completed.

15. EXPECTED RESULTS IN FY 1971

The projects listed under Item 12 above not completed in FY 1970 will be completed in FY 1971.

16. EXPECTED RESULTS IN FY 1972

Results in FY 1972 will depend upon the new investigatior. Work with Rock Valley mammals in connection with the irradiation source will continue. Sampling procedures will be used to correspond with needs of the IBP also. Some attention will be given to Plowshare activities.

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.:	4. Date Prepared:
06-02-04	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA and Nevada Test Site

7. Person in Charge:	8. Project Term:
Janice C. Beatley	From: 1962 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2 1/2	3	1 1/2
(b) Other Tech.	3/4	1/4	1/4
Total:	3 1/4	3 1/4	1 3/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 38,300	$ 41,000	$ 25,600
(b) Materials & Services	6,200	6,400	2,800
(c) Indirect Expenses *	25,400	24,900	17,200
Total:	$ 69,900	$ 72,300	$ 45,600

* 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 1970

Beatley, Janice C. Dependence of desert rodents on winter annuals and precipitation. Ecology 50: 721-724. 1969.

Beatley, Janice C. Biomass of desert winter annual plant populations in southern Nevada. Oikos 20: 261-273. 1969.

Reveal, James L. New species in Eriogonum and Gilia from southern Nevada. Bull. Torrey Bot. Club 96: 476-484. 1969.

12. SCOPE OF THE PROJECT

The program of establishment of biological and environmental baselines in selected ecosystems of the Nevada Test Site, designed as long-term studies and with objectives of enabling interpretation of effects of nuclear and other testing, was begun in 1962, and continues to the present.

13. RELATIONSHIP TO OTHER PROJECTS

Ecological and environmental studies conducted in desert regions of the world, and radiation effects studies on other AEC-DBM contracts, all have pertinence directly or indirectly to the plant, rodent, and environmental studies conducted at the Test Site.

14. TECHNICAL PROGRESS IN FY 1970

Ecology Studies. Collection of environmental data has continued on the 68 permanent study plots at the Test Site, for continuing characterization of the environments on each of the sites. Collections of other kinds of data, except where an additional year's record was wished, were discontinued.

NTS Flora. An additional 2300 plant collections, including 100 new taxa, from the Test Site, Nellis Air Force Ranges, and Ash Meadows, were accessioned for the Test Site herbarium, and duplicates distributed to various repositories in the country. The several new species are being described for the literature by monographers.

15. EXPECTED RESULTS IN FY 1971

All plot data collections will cease as of July 1, 1970, and the final year of field work will be devoted almost entirely to a completion of the plant collecting over the southern two-fifths of Nye County, Nevada (about 8000 sq. mi.). At the close of the collecting season in the fall, the final publication of the vascular flora will be prepared for publication. Preparation of this publication will take most of the remainder of this fiscal year.

Manpower during this fiscal year will consist of the investigator, a Lab. Tech. who will perform miscellaneous field and laboratory duties at the Test Site, an herbarium assistant for 6 months at the Test Site, and a half-time programmer in Los Angeles.

16. EXPECTED RESULTS IN FY 1972

The statistical analyses of the plant, animal, and environmental data

collected on the 68 permanent study sites during the years 1962-70, require a close working relationship with a biostatistician. If the assistance of a biostatistician is available, the investigator will devote full-time to the data analyses and preparation of manuscripts, through this fiscal year and beyond, until the conclusions regarding the interrelations among the variables, including the possible effects of ionizing radiation on the parameters measured, are forthcoming from the data, i.e., until the objectives of the program have been met.

If the services of a biostatistician are not available by the beginning of this fiscal year, the complex data analyses cannot be undertaken, and the investigator will request a half-time appointment until arrangements can be made for the completion of this 8-year study in an institution where the required assistance and facilities are available.

Personnel during this fiscal year will consist of the investigator and a half-time programmer.

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.:
06-02-04

4. Date Prepared:
April - 1970

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 Turner

8. Project Term:
From: 1961 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	3 1/2	2 3/4	3
(b) Other Tech.	1/2	1/4	1/4
Total:	4	3	3 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 56,600	$ 41,000	$ 49,800
(b) Materials & Services	4,000	5,000	5,000
(c) Indirect Expenses *	33,900	24,900	34,300
Total:	$ 94,500	$ 70,900	$ 89,100

* 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 1970

A demographic analysis of continuously irradiated and nonirradiated populations of the lizard, Uta stansburiana. Radiat. Res. 38: 349-356. (With P. A. Medica, J. R. Lannom, Jr., and G. A. Hoddenbach).

A demographic analysis of fenced populations of the whiptail lizard, Cnemidophorus tigris, in southern Nevada. SWest. Nat. 14: 189-202. (With P. A. Medica, J. R. Lannom, Jr., and G. A. Hoddenbach).

Estimating annual energy flow. Proceedings of Vertebrate Meeting, Utah State University, Sept. 18-19, 1969. 66 pp. mimeo., Appendices. J. Bowns, ed.

Density and composition of fenced populations of leopard lizards (Crotaphytus wislizenii) in southern Nevada. Herpetologica 25: 247-257. (With J. R. Lannom, Jr., P. A. Medica, and G. A. Hoddenbach).

Home ranges and body size of lizards. Ecology: in press. (With R. I. Jennrich and J. D. Weintraub).

The demography of the lizard, Uta stansburiana Baird and Girard, in southern Nevada. J. Anim. Ecol.: in press. (With G. A. Hoddenbach, P. A. Medica, and 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 investigated 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 were conducted 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 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

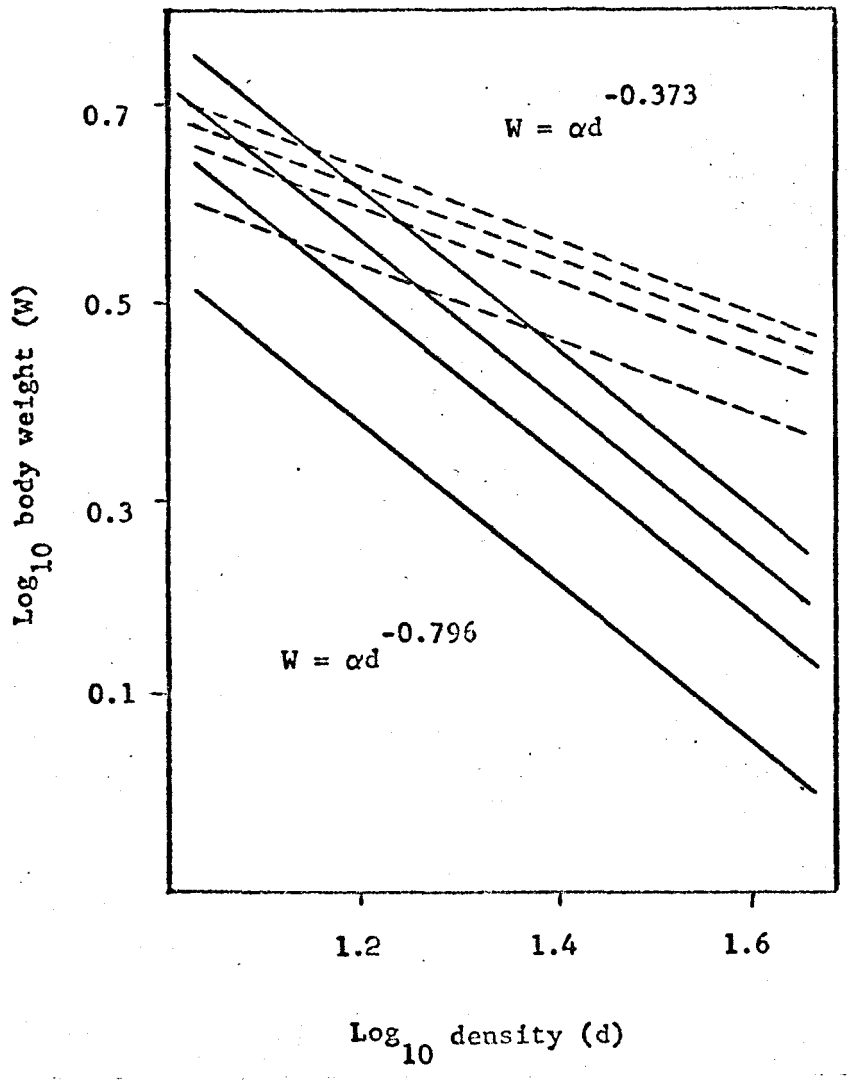
14. TECHNICAL PROGRESS IN FY 1970

1. Over a 3-year period, body weights (W) of the lizard Uta stansburiana, in the four study areas varied inversely with population density (d). These relationships were examined in terms of the following general model: $W = \alpha d^{\beta}$. Values of β for adults and juveniles were -0.373 and -0.796, respectively: There were also area and sex differences in the values of α.

Covariance analyses showed that egg clutch size was positively correlated with body size (weight and snout-vent length). These analyses indicated that, other factors being equal, a one gram decrease in body weight reduced clutch size by about one-third of an egg. It was also shown that extrinsic factors (e.g., temperature, precipitation) contributed far more to the explained variation in clutch size than the body size effect.

This last point should not detract from the potential importance of body size differences in regulating population density. The extrinsic factors are entirely independent of the state of the population. However, the body size effect on fecundity is responsive to changes in population size, and acts to reduce reproductivity in a dense population and enhance it in a sparse one.

2. Leopard lizards (Crotaphytus wislizenii) have been exposed to low levels of gamma radiation since January 1964. Average annual tissue doses, as measured by implanted lithium fluoride microdosimeters, have



been estimated at 400-500 rads/year. Demographic data, and the failure of mature female Crotaphytus in the irradiated area to assume the orange coloration typical of reproductively active females, indicated the absence of reproduction in 1967 and 1968. Three females from the irradiated plot in May and June of 1969 exhibited complete regression of the ovaries, undeveloped oviducal walls, and hypertrophied fat bodies. One of three irradiated males collected at the same time was apparently sterile. All of 20 control individuals taken in 1969 exhibited ovaries, oviducts, testes and epididymides normal for the season.

The lack of reproduction by irradiated Crotaphytus is attributed to female sterility induced by whole-body doses of approximately 1500 rads sustained between January 1964 and the spring of 1967. Reproduction by Uta stansburiana, in the irradiated area was apparently normal in 1966 and 1967. The difference in response of these two species is attributed to their markedly different life-spans and demographic regimes.

3. The relationship between home range, A (m^2) and body weight, W (g) among adults of 13 species of terrestrial lizards was estimated as: $A = 171.4W^{0.95}$. The slope of the logarithmic regression is

	Sex	Weights (g) Nonirradiated	Irradiated
Body	m	26.7 ± 1.40 (8)	26.2 ± 1.91 (3)
	f	47.9 ± 4.29 (12)	46.7 ± 1.32 (3)
Liver	m	0.43 ± 0.05 (6)	0.42 ± 0.05 (2)
	f	1.27 ± 0.18 (12)	1.40 ± 0.38 (3)
Kidneys	m	0.21 ± 0.02 (8)	0.23 ± 0.02 (3)
	f	0.23 ± 0.02 (12)	0.21 ± 0.03 (3)
Fat bodies	m	0.024 ± 0.015 (6)	0.133 ± 0.051 (3)*
	f	0.030 ± 0.013 (12)	1.93 ± 0.34 (3)**
Right testis	m	0.11 ± 0.015 (8)	0.16 ± 0.044 (3)

* t test significant at 5% level (t = 2.72)
** t test significant at 1% level (t = 12.30)

steeper than that relating standard metabolic rate, $\underline{M}$ (cm^3 O_2 per hour) at 30° C and body weight: $\underline{M} = 0.82\underline{W}^{0.62}$. These functions were compared with similar regressions relating the home ranges and basal metabolic rates of birds and mammals to body size. Two major difficulties in the interpretation of such data have been elucidated.

4. Six one-acre enclosures were constructed near Mercury (in the same area where work is now being conducted by Romney and Wallace). Densities and egg production of Uta in two of these enclosures were estimated during the 1969 season. One of the plots was specially irrigated during the late fall of 1969.

15. EXPECTED RESULTS IN FY 1971

1. Sampling of reptiles in the study areas in Rock Valley will be continued. This work will be directed towards the following objectives:

   a. documentation of the continued decline of leopard lizards in the irradiated plot, and the effects of the gradual removal of such a predator from the system.

   b. examination of reproductivity in whiptail lizards. One female removed from the irradiated plot in 1969 exhibited regression of ovaries and hypertrophy of fat bodies, and we believe that the females of this species may now be duplicating the experience of the female Crotaphytus.

c. confirmation of our published findings on Uta and its apparent immunity (through what might be termed "demographic protection") to continued gamma irradiation. Indirect effects, stemming from the disappearance of leopard lizards might, however, be expected.

d. periodic assessments of the standing crops of certain other species (e.g., larger predatory snakes, Chionactis, horned lizards, turtles) as a part of the validation site research projected in conjunction with the desert biome portion of the International Biological Program.

2. As staffing permits, work will be continued in the one-acre enclosures near Mercury. The research projects suited to these plots are what are called "process studies" in the desert and grasslands biome I. B. P. research designs. The purpose is to develop submodels of biological processes for various desert species. For the purpose of modellers constructing large system models the most important submodels are demographic ones (i.e., predictive expressions of rates of reproduction and mortality in natural populations as a function of some array of independent variables). The development of such models requires that the investigator subject enclosed populations to various experimental combinations of physical and biotic factors.

3. Work on models of lizard bioenergetics will be continued. The approach is fairly well illustrated in a short presentation prepared for a meeting of vertebrate specialists in Logan, Utah, in September 1969. (See Reference 3, in section 11).

16. EXPECTED RESULTS IN FY 1972

The program in FY 1972 will be largely focused in Rock Valley, as before, with the major problems being:

a. coordination of the sampling of populations of plants and animals, both to continue our investigations of the fate of the irradiated community and to acquire appropriate data for our I. B. P. validation site

b. maintenance of the meteorological monitoring system prescribed by the I. B. P. design, with the regular retrieval and reduction or pertinent meteorological variables. Particular emphasis needs to be placed on summation procedures so that complex arrays of meteorological data are reduced to more manageable variables (e.g., day-degrees above some specified threshhold).

c. maintenance of readily retrievable data sets, and the regular transmission of all pertinent information to the I. B. P. modelling team in Logan, Utah

d. active participation in the development of the desert biome model by means of consultations with the mathematicians and other members of the modelling team

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.:
06-02-04

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	6	5	5
(b) Other Tech.	1/4	1/4	1/4
Total:	6 1/4	5 1/4	5 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 78,800	$ 61,900	$ 63,000
(b) Materials & Services	9,300	10,300	10,300
(c) Indirect Expenses *	50,800	41,500	34,300
Total:	$ 138,900	$ 113,700	$ 107,600

* 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 1970

The isolation of manganese in a system between Bis (2-ethylhexyl) Hydrogen Phosphate and Aqueous Nitric Acid J. Inorg. and Nucl. Chem. (In Press).

12. SCOPE OF THE PROJECT

The primary objectives of the Chemical Problems Section are to maintain a highly diversified analytical and radio-chemistry facility in support of the various research programs of the Environmental Radiation Division. The second objective is to develop and maintain a current up-to-date radioassay and analytical instrumentation facility to support the ever-expanding needs of the Division's research programs. The third major objective is to carry on continuous research studies in the development of new analytical techniques both in instrumentation and analytical chemistry with particular emphasis given to ecological oriented problems.

In addition, this Section is involved in a number of other research programs. The behavior of water extractable elements in soil as a function of high gamma and/or beta radiation is being studied. Environmental fission product fallout profiles in the Los Angeles basin are being maintained, and have been maintained, for the past eleven years.

Research is continuing in the development of a rapid method for the accurate estimation of age of field-collected rodents and mammals using neutron activation analysis techniques. This approach is based upon the fact that calcium is continuously deposited in the dentin matrix of the teeth throughout the life span of the animal and thus there should be an accurate relationship between age and calcium concentration in the dentin.

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, Tennessee.

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

Lawrence Radiation Laboratories, Livermore, California.

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 1970

During the first eight months of FY 1970 this Section in supporting division research programs completed 2000 determinations of Sr^{90}, Ce^{144},

Cs^{137}, Mn^{54}, Br^{82}, Cs^{134}, Ba^{140} and the stable elements of Ca, Mn, O, K, Th, and U in plant, soil, milk, bone, vital organs and gastro-intestinal tracts samples. In addition, 650 plant, soil, and various animal tissue samples were analized for mixed fission products by gamma pulse height analysis. Three hundred plant samples were analized for stable Cs by activation techniques.

15. EXPECTED RESULTS IN FY 1971

It is expected the support work of division programs now in progress will continue at the same level for the remaining fiscal year. However, based upon projected division research programs for FY 1972 this Section's support activities will shift from fission product analysis to stable elements analysis. The existing gamma spectrometry facility will amost entirely support fission product cycling programs now in progress.

16. EXPECTED RESULTS IN FY 1972

The soil irradiation study is expected to be completed sometime during the last half of FY 1972. No major changes in the Section's support activities are anticipated, although, all wet chemical analysis for gamma emitting radionuclides will have been terminated and only pure beta or alpha emitting isotopes will be analized by wet chemical analysis.

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.:	4. Date Prepared:
06-02-04	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA

7. Person in Charge:	8. Project Term:
O. R. Lunt	From: 1966 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	1	1	1
(b) Other Tech.	1/4	1/4	1/4
Total:	1 1/4	1 1/4	1 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 15,400	$ 16,700	$ 16,700
(b) Materials & Services	2,300	3,300	3,800
(c) Indirect Expenses *	8,500	8,300	8,600
Total:	$ 26,200	$ 28,300	$ 29,100

* 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 1970

None.

12. SCOPE OF THE PROJECT

Soil-water-plant relations: Work is in progress with Dr. John Letey of UC Riverside participating on transpirational and photosynthetic activity of Mojave Desert species as a function of soil moisture suction. These studies are expected to provide insight on physiological processes of plants having extreme draught tolerance and contribute to an understanding of plant distributions in certain locations under study at the Nevada Test Site.

The determination of oxygen diffusion rate requirements of three species suspected of showing soil textural sensitivity in their distribution has been completed. Further work along this line has been discontinued in favor of water relations work.

Mineral nutrition studies: Tolerance of several Mojave Desert species to soluble salts and certain specific ions was investigated through a graduate student. Nitrogen input to the ecosystem is being studied with Dr. Romney using the acetylene reduction method. Calcium studies are concerned with the effect of this ion on active transport.

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 1970

Research done on oxygen requirements of roots of Artemisia tridentata, Larrea tridentata and Franseria dumosa. The order of tolerance to low oxygen supply was Franseria >> Larrea > Artemisia. These data may provide a physiological basis for some distributions of the species on certain soils. The data are being prepared for publication.

Continued work on nitrogen fixation implicates fixation in connection with Bromus rubens, a grass. This observation invites further investigation. As would be expected, several legumes display fixation.

Equipment difficulties seem to be resolved in doing transpiration-net CO_2 assimilation studies, and studies on transpiration as related to soil moisture suction are now under way.

15. EXPECTED RESULTS IN FY 1971

Principal attention will be given to studies on water relations and comparative studies among desert species in this regard.

16. EXPECTED RESULTS IN FY 1972

Focus in FY 1972 is expected to be on moisture relations and in the area of mineral nutrition. Increasing attention will be directed to questions bearing on mineral cycling. Because of the limited size of the budget on this program and the heavy administrative duties of the principal investigator, it is more difficult than is usual to project results much more than a year in advance.

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.:
06-02-04

4. Date Prepared:
April - 1970

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 A. Wallace (Acting)

8. Project Term:
From: 1971 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	1	3	4
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total:	$1\frac{1}{4}$	$3\frac{1}{4}$	$4\frac{1}{4}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 19,300	$ 44,300	$ 54,200
(b) Materials & Services	3,200	3,400	4,400
(c) Indirect Expenses *	8,500	33,200	34,300
Total:	$ 31,000	$ 80,900	$ 92,900

* 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 1970

Koller, D., and Kigel, J. The Growth of Leaves and Tillers. Agronomy Journal (In press).

Koller, D. The Partitioning of Resistances to Photosynthetic CO_2-Uptake in the Leaf. UCLA 12-743. 1969.

12. SCOPE OF THE PROJECT

Several ecologically important aspects of plant productivity, plant characteristics, soil-plant water relationships are to be evaluated with null-point gas exchange equipment which has been partly developed in this laboratory. The principle upon which the technique operates is feed back controls which maintain a small chamber either at ambient conditions or at predetermined conditions of temperature, CO_2 and water vapor contents or even at other varied conditions. These special studies are of considerable importance in the ecosystem studies being done in the division.

This equipment will be used in the field to help assess primary productivity by making measurements of apparent net photosynthesis and respiration from which, in conjunction with other measurements to be made simultaneously, root and stem growth of perennial plants can be estimated. Other measurements to be made include direct excavation of some plants and C^{14} distribution in these and others.

Other uses of this equipment include a study of soil moisture, and photosynthesis. Leaf water, leaf dry weight and leaf temperature will be simultaneously measured. Transpiration would automatically also be measured.

Photosynthesis and transpiration measurements will be made on a 24-hour basis for several species to determine some fundamental characteristics of the plants. Respiration will also be calculated.

The compensation point can be determined for each of the several species by turning off the CO_2 input and measuring the point at which the CO_2 levels off. Oxygen inhibition can also be measured on the same species by replacing the air line with tanks having different percentages of oxygen - 10, 20, 50 and 80%.

Effects of the irradiation source in Rock Valley on transpiration, net photosynthesis, respiration, and succulence will subsequently be studied.

A large majority of these studies will be in the field and directed toward ecosystem studies and irradiation effects.

The staff in this section will also have much responsibility for studies of plant community dynamics.

The new staff member appointed in this area most likely will have interests and capability in modeling of ecosystems. Should such a person be successfully appointed he will use a synthetic approach to ecosystem analyses and will collaborate with other members of the division and with the International Biological Program Desert Biome researchers from other universities.

13. RELATIONSHIP TO OTHER PROJECTS

Research is correlated with other sections of the Environmental Radiation Division: Plant Physiological Ecology, A. Wallace; Plant Factors, E. M. Romney; Physiological Ecology, Jack Dainty; Desert Ecology (Nevada Test Site Ecology), J. C. Beatley; Environmental Factors, H. A. Hawthorne; Physiology of Mineral Accumulation in Plants, O. R. Lunt.
University of California, Riverside, California, J. Letey
Utah State University, Logan, Utah, David Goodall
University of Nevada, Las Vegas, Nevada, F. Went
Battelle-Northwest, Hanford, Washington, W. H. Rickard

14. TECHNICAL PROGRESS IN FY 1970

The Siemens null-point gas exchange equipment was assembled. Revisions were made and the equipment was automated and tested.

15. EXPECTED RESULTS IN FY 1971

Carbon dioxide exchange (photosynthesis) and water exchange (transpiration) studies will be conducted to evaluate many aspects of basic plant ecology as well as aspects relating to irradiation effects. Emphasis will be on primary productivity and energy exchange.

Synthetic modeling studies of the desert ecosystem will most likely be undertaken with some emphasis on mineral and radionuclide cycling.

16. EXPECTED RESULTS IN FY 1972

Studies will be continued and expanded. Efforts will be made to obtain information useful to both the AEC and International Biological Program.

Should appointment be successful of a new staff member with interests and capability in computer modeling, these studies would be greatly expanded in this Fiscal 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:
Physilogical Ecology

3. AEC Budget Activity No.: 06-02-04

4. Date Prepared: April - 1970

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

6. Working Location: UCLA

7. Person in Charge: Jack Dainty

8. Project Term: From: 1970 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	1	½	½
(b) Other Tech.	¼	¼	¼
Total:	1 ¼	¾	¾

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 16,900	$ 7,900	$ 7,900
(b) Materials & Services	3,400	3,300	3,300
(c) Indirect Expenses *	8,500	8,300	8,600
Total:	$ 28,800	$ 19,500	$ 19,800

* 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 1970

None

12. SCOPE OF THE PROJECT

I intend to study two aspects of the physiology of desert plants. One of these is the physiology of drought-resistant leaves. There are two major problems here which interest me: the water relations of such leaves and, in the appropriate species, the salt-excreting properties. Many drought-resistant leaves are said to possess water storage and buffering systems such as vesicles, other special cells, thick cell walls, etc. The way in which these systems can act to store and release water, depending upon conditions, is quite obscure and I would like to investigate this problem, perhaps with Atriplex species, both by theoretical and experimental methods. Salt-excreting glands occur in the leaves of such species as Tamarix. I propose a fundamental investigation of these using electrophysiological techniques.

The other major aspect of physiology which interests me is ion uptake by desert plants. Most, perhaps all, ion uptake studies with plant have not been made with ecological considerations in mind. There are almost no quantitative studies on 'natural' whole plants. I propose to initiate such a study to try and obtain such a parameter as the rate of uptake of various ions, per unit area of root, as a function of ionic concentration, distance from the root apex, water potential of the surrounding medium, etc. This work will have to start using seedlings in water culture and gradually proceed to mature plant in natural situations.

13. RELATIONSHIP TO OTHER PROJECTS

Relevant work is going on in Oxford, East Anglia, Sheffield and Aberdeen in the U. K., in Canberra Perth, Sydney and Adelaide in Australia, and in Riverside in the U. S. A.

14. TECHNICAL PROGRESS IN FY 1970

Some initial work on the anatomy of drought-resistant leaves is taking place.

15. EXPECTED RESULTS IN FY 1971

A great deal of clarification of the water relations of leaves is expected. Progress will be slower on salt-excreting glands and on ion uptake by whole plants.

16. EXPECTED RESULTS IN FY 1972

Further clarification of leaf water-relations will be achieved. Electrophysiological work on salt-excreting glands should be showing results. Ion uptake studies on whole plants will still be slow; the problem is so enormous.

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 Survey and Characterization

3. AEC Budget Activity No.:
06-02-04

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA and Nevada Test Site

7. Person in Charge:
Verle Q. Hale (Acting)

8. Project Term:
From: 1970 To: 1973

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	0	1	1
(b) Other Tech.	0	1/4	1/4
Total:	0	1 1/4	1 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 0	$ 18,900	$ 20,000
(b) Materials & Services	0	2,300	2,500
(c) Indirect Expenses *	0	8,300	8,600
Total:	$ 0	$ 29,500	$ 31,100

* 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 1970

None

12. SCOPE OF THE PROJECT

The major goal of the soil evaluation studies is to provide the background information for an ecosystem study or series of ecosystem studies. Soils descriptions and their chemical and physical properties are being determined. It is expected that a detailed soils map will be made of Rock Valley as well as less detailed maps of other areas at the Nevada Test Site where ecological studies are being made by the division. All these soils studies are made in support of the ecological studies and the purpose is to know the soil characteristics as one of the environmental components of the ecosystems. It is hoped that soil series names can be placed on the soils and studies now being directed toward that goal where appropriate, radionuclide distribution in profiles is being determined in areas subjected to past nuclear testing.

The results being obtained are also being translated into data for components of the nitrogen and phosphorus cycle in the ecosystem studies and sufficient relevant data are being taken to make the cycles more complete. N^{15} and C^{14} are to be used to evaluate decomposition rates. Simultaneous data are also being taken to give information on K, Mg, Ca, Zn, Cu, and Mo cycling. These data are important in the interpretation of those obtained in the radionuclide cycling studies.

13. RELATIONSHIP TO OTHER PROJECTS

Related studies are conducted at the following locations:

University of Nevada, Reno, Nevada.
International Biological Program, Utah State University, Logan, Utah.
Southwest Radiological Health Laboratory, Las Vegas, Nevada.

14. TECHNICAL PROGRESS IN FY 1970

Descriptions of 76 profiles have been completed and chemical and physical analyses of many of these will also be completed. Profiles were photographed. Leaves of plants near each profile were collected for mineral analyses.

15. EXPECTED RESULTS IN FY 1971

Some more pits will be excavated particularly in Rock Valley and also in connection with more of Dr. Beatley's plots. Some emphasis will be on the northern parts of the Nevada Test Site also. Distribution of radionuclides in profiles from past nuclear testing will be studied. Phosphorus and nitrogen cycling models will be developed from soil and plant analyses. Behavior of organic matter and litter in profiles will be explored with N^{15} and C^{14}.

16. EXPECTED RESULTS IN FY 1972

Studies will be continued and completed where possible. The number of soil pits will be expanded if prior work indicates. It is expected that the entire soils description and analyses will be published in a single volume or as otherwise needed for ecosystem studies in irradiation effects evaluation.

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:
Distribution and Interrelationship of Elements in Biological Systems

3. AEC Budget Activity No.:	4. Date Prepared:
06-02-04	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA

7. Person in Charge:	8. Project Term:
George V. Alexander	From: 1970 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	0	1	1
(b) Other Tech.	0	-	-
Total:	0	1	1

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 0	$ 14,300	$ 14,300
(b) Materials & Services	0	1,000	1,500
(c) Indirect Expenses *	0	8,300	8,600
Total:	$ 0	$ 23,600	$ 24,400

* 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 DURING FY 1970

None.

12. SCOPE OF THE PROJECT

The primary research goal of this project is to study the distribution and interrelationship of the elements present in biological systems. Of fundamental importance to this goal is the development of efficient analytical methods which will produce accurate results. In an attempt to meet these requirements and emission spectrometric analytical system has been developed which requires little sample preparation, is simple to operate and is capable of determining some 25 elements commonly observed in plant and animal tissues. This system is being used for routine analysis at present, but, with the addition of several refinements, will remain in a developmental state for several years to come.

13. RELATIONSHIP TO OTHER PROJECTS

Research activities will be associated with other sections of the Environmental Radiation Division notably E. M. Romney (Plant Factors), A. Wallace (Plant Physiological Ecology), O. R. Lunt (Physiology of Mineral Accumulation in Plant), H. Hawthorne (Environmental Factors) and H. Nishita (Soil Factors).

14. TECHNICAL PROGRESS IN FY 1970

By far the largest amount of effort has been devoted to the analysis of over 11,000 plant samples during the past year. For the most part these samples are related to the studies of element distribution and translocation in desert plants of the Nevada Test Site Region.

Studies have been carried out to improve the overall performance of the spectrometric system. The method for calibrating the system for the various parameters necessary to reduce the observed signals to elemental concentrations has been significantly improved.

15. EXPECTED RESULTS IN FY 1971

The major task for FY 1971 will be to correlate and summarize the many observations made during this past year in order that we can more intelligently continue or redirect our future analytical efforts.

16. EXPECTED RESULTS IN FY 1972

Studies of the distribution and interrelationship of elements in biological systems will be continued in directions indicated by past results and by the interests of collaborators. Methods will be developed and improved as necessary to meet these needs.

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.:
06-02-08

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	2 ½	1 ½	1 ½
(b) Other Tech.	-	-	-
Total:	2 ½	1 ½	1 ½

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 33,000	$ 26,400	$ 26,800
(b) Materials & Services	2,000	2,000	2,000
(c) Indirect Expenses *	17,000	16,600	17,200
Total:	$ 52,000	$ 45,000	$ 46,000

* 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 1970

Publications relative to applications of instrumentation for characterization of cells is given under Budget Activity 06-01-01.

12. SCOPE OF PROJECT

The purpose of this Medical Physics Section activity is to develope 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 Instrumentation). 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 on in vivo and in vitro irradiations of lymphocytes on these newly measurable characteristics are being determined (See 14 below) and the results discussed in Schedule 189 for budget 06-01-01.

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 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 1970

High Speed Section Scanner: The high speed section scanner using a spherical cap nest of seven 3-inch crystals with 7 fine grained collimators all lined up accurately to a common focus, has been continued to be used in a clinical program of fast section brain scanning in cooperation with Dr. Paul Crandall of the UCLA Neurosurgery Division. Technical and instrumental improvements have been incorporated enabling better quality and higher resolution scans to be obtained. The hydraulically controlled antiscalloping system has been of great help in this respect. An even better anti-scalloping system is being evolved which also eliminates time-constant distortion and could have a much greater range of applicability in nuclear medical dynamic studies.

The application of fast section scanning to pulmonary and liver scanning has been temporarily delayed on account of building modifications in the UCLA School of Medicine. The program will very soon be continued. Eight liver section scans had been obtained on patients. These scans yield much more diagnostic information than conventional liver scans.

Although this scanning system is not a simultaneous multiplane tomographic scanner it is much simpler and fast enough to make several sections in a reasonably short patient set-up time.

Physical Cell Measurements and Separations: The systems developed for (a) precision density gradient separation methods (b) use of cyto-centrifuge for planting dilute cell suspensions on slides (c) Coulter aperture cell size determinations (d) electrophoretic cell separations in which the cells are maintained in an almost normal environment, were used in a productive program some of which is described under 06-01-01. Frequent technique and instrumentation improvements have been incorporated which have increased the reliability of the results obtained and have speeded up the acquisition of useable data.

After delays arising from personnel changes the program to obtain flash holographic micrographs of cells of larger than a predetermined size, as sensed by a Coulter aperture, is now under way.

15. EXPECTED RESULTS IN FY 1971

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 1972

It is anticipated that "cross-time" correlation scanning system can be developed.

If new improved scintillators or high atomic number solid state scinillators or high atomic number solid state detectors become available commercially, it is anticipated that they can be incorporated into improved nuclear medicine imaging systems. It is expected that new methods of physical "cytometry" can be evolved.

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.:	4. Date Prepared:
06-03-01	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA

7. Person in Charge:	8. Project Term:
George V. Taplin, M.D.	From: 1958 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	4 3/4	5 3/4	5 3/4
(b) Other Tech.	1/4	1/4	1/4
Total:	5	6	6

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 70,000	$ 84,100	$ 85,300
(b) Materials & Services	13,700	9,100	9,700
(c) Indirect Expenses *	42,400	49,800	51,500
Total:	$ 126,100	$ 143,000	$ 146,500

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

11. PUBLICATIONS DURING FY 1970

Taplin, G.V., Poe, N.D., Dore, E.K., Swanson, L.A., Isawa, T., and Greenberg, A. Scintiscanning and Roentgenographic Procedures In Managing Major Pulmonary Disorders, Medical Radioisotope Scintigraphy, Proceedings of the Symposium held by the International Atomic Energy Agency in Salzburg, August 6-15, 1968. Vol. II, 111-124, July, 1969.

Isawa, T., Wasserman, K., and Taplin, G.V. A Variability of Lung Scans Following Pulmonary Embolization, American Rev. of Respiratory Diseases, (In Press).

Taplin, G.V.,Dore, E.K., Poe, N.D., Swanson, L.A., and Greenberg, A. Pulmonary Arterial Perfusion and Aerated Space Assessment by Scintiscanning, Frontiers of Pulmonary Radiology, Harvard Univ. Symp. April, 1967, Grune-Stratton, Publishers. 2:33-75, Oct. 1969.

Isawa, T., Benfield, J.R., Johnson, D.E., and Taplin, G.V. Lung Scanning and Differential Bronchospirometry: A Comparative Study, Surgical Forum, 6: 230-231, Oct. 1969.

Taplin, G.V. Scintiscanning in the Assessment of Regional Pulmonary Function, Clinical Cardiopulmonary Physiology, Third Edition, Section II, No. 10, 437-465, Grune and Stratton, publishers, Gordon, Carleton and Faber, editors, January, 1970.

Taplin, G.V., Dore, E.K., Poe, N.D., Swanson, L.A., and Greenberg, A. Lung Perfusion and Aerated Space Assessment by Scintiscanning, USAEC conf. 670958, 281-309, 1969. Proceedings of the Symposium in Nuclear Med. - Its Current Status in Medical Practice, Cleveland, Ohio, Sept. 27-30, 1967.

Poe, N.D., Dore, E.K., Wilson, A.F., and Taplin, G.V. Inhalation-Perfusion Lung Scanning in Diagnosing Pulmonary Embolism, J. of Nuc. Med. 10: 427, 1969.

Rodriguez, J., MacDonald, N.S., and Taplin, G.V. Preparation of 113m Indium Albumin Aggregates for Lung and Liver Scanning, J. of Nuc. Med. 10: 368, 1969.

Yamada, H., Johnson, D.E., Griswold, M.L., and Taplin, G.V. Radioalbumin Microaggregates for Reticuloendothelial Organ Scanning and Function Assessment, J. of Nuc. Med., 10:453-454, 1969.

Yamada, H., MacDonald, N.S., and Taplin, G.V. Factors in Improving Yield and Quality of Technetium 99m Labeled Serum Albumin, J. of Nuc. Med. 10:454, 1969.

Isawa, T., Wasserman, K., and Taplin, G.V. Lung Scintigraphy and Pulmonary Function Studies in Obstructive Airways Disease, J. of Nuc. Med. 10:345, 1969.

Taplin, G.V. The Scintillation Scan, Clinical Scintillation Scanning, ed., Freeman and Johnson, 5:119-157, Hoeber Medical Division, Harper & Row, Publishers Inc., 1969.

12. SCOPE OF THE PROJECT

The major goal is to develop new applications of radionuclides and nuclear technology in medical practice. Such radioisotope procedures should reveal reliable information which is otherwise impossible, impractical or hazardous to obtain or they should supply supplementary data which aid evaluation of other diagnostic procedures. Organ imaging techniques are unique in that they permit nontraumatic measurement of tracer as it enters or leaves internal organs. Furthermore the size, shape and position of internal organs may be visualized and abnormalities such as tumors, cysts or abscesses may be detected as areas of either increased or decreased tracer concentration. With recent developments of rapid imaging devices dynamic studies of organ function and blood flow become feasible as practical clinical test procedures.

During the past year clinical investigations have been conducted to further clarify the role of organ scintigraphy in such clinical problems as the diagnosis and management of pulmonary embolism, the detection and treatment of the rejection phenomenon in kidney transplantation, the differential diagnosis of medical versus surgical jaundice, the classification of chronic obstructive lung disease, experimental lung transplantation and reimplantation and dynamic studies of the heart, liver and reticuloendothelial system.

The planned consolidation of the nuclear medicine facilities of the laboratory and those in the medical center have been delayed because the anticipated expansion of the hospital facilities to 1,000 beds has progressed much more slowly than originally anticipated. Likewise the dates for receipt and operation of the biomedical cyclotron have been moved forward approximately one year to July and September, 1970 respectively. However, two professional members of the division have moved to the medical center and are helping in the development of a closer coordination of the laboratory's nuclear medicine research with similar activities in the Department of Radiology. It is anticipated that the move to the medical center will be completed during the summer and early fall of 1970. It is likely that the laboratory's clinical research efforts will be continued at Harbor General Hospital at about the current level for the next two years or until equal facilities for clinical studies become available in the medical center.

13. RELATIONSHIP TO OTHER PROJECTS

Similar clinical applications of radionuclides and nuclear technology are being made at many of the major universities and medical centers in the United States such as at Johns Hopkins University under Doctor Henry N. Wagner, at the University of Pennsylvania under Doctor David Kuhl, at the Mayo Clinic under Doctor W. Newlon Tauxe, at Argonne National Laboratories in Chicago under Doctor Alexander Gottschalk and Doctor Paul Harper, at Northwestern University and Wesley Memorial Hospital under Doctor James Quinn III, at the Ochsner Clinic in New Orleans, Louisiana under Doctor William Maxfield, at the Mallinckrodt Institute of Radiology under Doctor James E. Potchen, at the University of Miami, Florida under Doctors Alfred Gilson and Edward Smith and at the University of California, Donner Laboratories under Doctor John H. Lawrence. Similar studies are also being conducted in various foreign medical centers such as at the University of Heidelberg, West Germany, under Doctor Kurt Scheer, at the University of Athens, Greece under Doctor Malamos, at the University of Pisa, Italy under Doctor Luigi Donato, in the Mexican Atomic Energy Agency and Institues of Cardiology and Neurology by Doctor Roberto Maas, at the Institute of Radiological Sciences, Chiba, Japan under Doctor Nagai, at the Imperial University of Tokyo under Doctor Hideo Ueda,

at the University of Kanazawa, Japan under Doctor Hiramatsu and at Guys Hospital, London and the French Atomic Energy Agency at Dorsay, France.

14. TECHNICAL PROGRESS IN FY 1970

Lung Scintigraphy Studies: During the past year and one half, with the services of Doctor Toyoharu Isawa (Dernham Foundation Senior Fellow) working in the Radioisotope Division at Harbor General Hospital, particular emphasis has been placed on the study of chronic obstructive pulmonary disorders by radioisotope scintigraphy (lung perfusion, radioaerosol and radioxenon gas inhalation scanning) in conjunction with standard tests of pulmonary function through the cooperation of Doctor Karl Wasserman, Chief of the Pulmonary Disease Division of the Department of Medicine. During this time scanning was facilitated by the availability of a 10 probe rectilinear scanner and during the past ten months lung inhalation studies with 133 Xenon gas became possible with the acquisition of an Anger scintillation camera. The three lung imaging procedures have now been employed in studying thoroughly nearly one hundred patients with obstructive airway disease. Three tentative, but potentially significant conclusions can be drawn regarding the aerosol inhalatinn scan. First, this procedure appears to be more sensitive than routine lung function tests in detecting airway abnormalities such as in chronic bronchitis and in asthmatic patients between attacks. Secondly, the aerosol scan patterns in chronic obstructive lung disease show two distinctly different configurations and combinations of the two. These patterns correspond with the major two types, emphysematous and bronchitic, as described by Burrows and Fletcher. More importantly in patients with features of both types the bronchitic component is readily detectible. The capacity to recognize bronchitic features is highly important because the bronchitic component is more readily amenable to treatment than pure emphysema. Thirdly, in studies of normal and airway disease patients abnormalities of lung clearance mechanisms become readily apparent when scans are made repeatedly during the first few hours following radioaerosol inhalation. Studies of this type should be helpful in better characterizing the nature and location of abnormal airway clearance mechanisms in chronic obstructive lung disease and in bronchogenic carcinoma. Serial lung imaging after aerosol inhalation appears to be a potentially valuable indicator of the patient's response to medical treatment in chronic lung disease and may also be equally useful in evaluating the effectiveness of aerosolized mucolytic, antibotic, and bronchodilator agents. Studies of this type might well be a useful adjunct to the study of uranium miners at the University of Colorado. A joint effort is now in the planning stage to coordinate our radioaerosol studies with other investigations under Doctor Donald Brown in Colorado and with Dr. Paul Morrow of the University of Rochester.

Other Lung Scintigraphy Studies: Work has continued on the problem of diagnosing pulmonary embolism in middle aged or older patients having associated chronic cardiopulmonary disorders. The great majority of our requests for lung scans at Harbor Hospital pose this problem. In the 30-40 percent of patients without complicating cardiopulmonary disease, pulmonary embolism may be diagnosed with a high degree of probability when the chest film is negative and the perfusion scan shows multiple areas of ischemia. On the other hand with underlying chronic obstructive lung disease the regional ischemia may be secondary to airway obstruction. It is under these conditions that the perfusion scan alone is not sufficiently reliable. The aerosol or radio-xenon inhalation examination is needed to demonstrate whether the ischemic region is ventilated or not. The combined perfusion-inhalation scan has

helped confirm the diagnosis of embolism or to exclude such a diagnosis in approximately half of this group. In the first seventy five patients so studied the inhalation scans were performed one to three days later. Currently both procedures are performed within an hour of each other by using aggregates labeled with 99m Tc and albumin aerosol with Indium-113m. The latter combination should give better results because the patients are studied for both functions almost simultaneously.

Liver and RES Studies: Doctor Hideo Yamada completed his stay with us at Harbor Hospital at the end of September, 1969. One of his major contributions, namely Sequential Liver and Upper Abdominal Scanning with Radio rose bengal " was completed. Such studies were found to be particularly useful in distinguishing medical from surgical jaundice with patients with acute problems. In many instances he was able to demonstrate abnormal accumulation of radioactivity in the extra as well as intrahepatic bile ducts during the first few hours following rose bengal injection. Such findings were also accompanied by delayed appearance of the dye in the upper intestine but by a few hours intestinal entry of dye occurred indicating partial rather than complete obstruction. On the other hand in cases of jaundice of two weeks or more duration, similar distinguishing features occurred but required repeated scan examinatinns for as long as three to five days.

The other facet of Doctor Yamada's research was in the improvement of labeling of albumin with 99m technetium and in the preparation of micro-aggregated technetium albumin suspensions of uniform size in the 1-3 micron range. Results of these studies were reported in the June issue of the Society of Nuclear Medicine Journal, 1969.

Doctor Kenichi Kitani has taken Doctor Yamada's place and is now working on the evaluation of reticuloendothelial system functions using 99m labeled microaggregates. Presently he is studying the metabolism of this agent and its turnover rates in the liver, bone marrow and spleen by sequential camera imaging of these organs. In control studies he is showing the difference in metabolism of iodine versus 99m Tc labeled albumin particles. Such controls are necessary because once the label is broken off each of the two elements has its own metabolic pathway. Another control study involves the demonstration of the effects of particle size variations in the test material. Preliminary clinical trials have shown tremendous differences between normal subjects versus those with acute infections, acute hemolytic anemia and Hodgkins disease. Similar studies are planned for patients with various malignancies in an attempt to show a possible relationship between RES functions and tumor growth or spread. Another aspect of the RES research is a collaborative project with the surgery group under Doctor Moore in studying the rejection phenomenon in canine kidney transplantation. Here it will be interesting to follow spleen size and function before, during and after rejection. The RES studies will be correlated with the results of Doctor Michael Hayes with radioisotope kidney function studies using the new Picker Dynacamera with its dual isotope and area of interest capacity.

Studies of the Liver's Dual Circulation by Hideo Yamada, P. Ramanathan and G.V. Taplin: The dynamic aspects of the liver's dual circulation were studied with both the Magnacamera and the Dynacamera following rapid intravenous injection of 99m Tc albumin solutions or microaggregates. Studies were made in normal subjects with patients with space-occupying lesions and in liver cirrhosis. Areas of normal versus abnormal liver (cold lesion) were identified

during replay of the video tape. Analogue tracings from each of the two areas were obtained with"cursors" during the first sixty seconds and again at five minutes following injection.

Normally the hepatic arterial curve begins to rise one to two seconds after the peak of the left heart curve. It reaches a plateau in about five seconds and is followed by more gradual rise which is considered to represent the portal venous compnnent. In the tumor region, the arterial component rises to a plateau quickly but is not followed by a further increase in radioactivity during the usual portal phase, thereby indicating a diminished or absent portal flow. In advanced cirrhosis the arterial component is more prominent than in normal subjects but the portal venous phase is reduced and sometimes absent. This capability permits one to study alterations in the arterial and venous components of the liver circulation both in parenchymal disease as well as in localized liver lesions such as cysts, primary and secondary tumors. The procedure deserves further investigation because of its potential usefulness in the management of cirrhosis and its possible diagnostic value in distinguishing malignant from benign lesions of the liver.

15. EXPECTED RESULTS IN FY 1971

Lung Function Studies: Regional lung function studies will be continued by Doctor Isawa in collaboration with Doctor Karl Wasserman of the Pulmonary Function Laboratory and Pulmonary Disease Section of the Department of Medicine at Harbor Hospital. Long term follow-up studies will be continued in patients with chronic obstructive pulmonary disease and bronchogenic carcinoma. Emphasis will be placed on sequential radioaerosol inhalation scanning to provide better information on lung clearance mechanisms in normal subjects as well as those with obstructive airway disease and also for the purpose of evaluating the patient's response to therapy in emphysema patients with a bronchitic component. More emphasis will also be placed on studying ventilatory abnormalities using the new wide field scintillation camera and its attachments for quantifying regional changes of lung ventilation. Furthermore, study of chronic obstructive pulmonary disease patients will be made to help classify them into types which have reversible versus irreversible disease. We hope to accomplish this classification by correlating function improvement as measured by spirometric tests before and after bronchodilators with structural as well as functional changes disclosed by scanning procedures and radiography. Other lung function studies will include the continuation of the lung transplantation and reimplantation work begun in FY 1970 along with studies of experimental emphysema in dogs induced by inhalation of papain aerosols.

RES Studies: Work in this area will be continued by Doctor Kitani. It is hoped that during this year a useful and reproducible method for studying the digestive capacity of the reticuloendothelial cells in the liver, spleen and bone marrow may be developed. Furthermore it is hoped that visualization of the organ turnover rates of radiocolloids which are susceptible to proteolytic digestion will reveal abnormalities in the turnover rates in each of the three major organs of this system and that such findings may have diagnostic and/or prognostic usefulness in the management of the wide variety of diseases which involve this system.

Kidney Function Studies: Work in this area will be continued by Doctor Michael Hayes as a consultant to the laboratory and as the person in charge

of scanning studies at Harbor General Hospital. Doctor Hayes plans to continue his collaborative studies with Doctor Moore in investigating the rejection phenomenon in renal transplantation in dogs as well as in patients. In these studies he will be using the large crystal scintillation camera which makes possible the quantitation of sequential renal scans in various kidney disorders along with the use of dual isotopes to study the relations between renal blood flow and filtration simultaneously with radionuclide compounds of widely different energy spectra.

16. EXPECTED RESULTS IN FY 1972.

Lung Studies: The various types of lung function studies conducted during FY 1971 are likely to be continued by Doctor Isawa again in collaboration with Doctor Karl Wasserman of the Pulmonary Function Laboratory. Emphasis will be continued on developing better methods for evaluating abnormalities of lung clearance mechanisms. Also new procedures and tracer materials will be studied in an attempt to develop a useful method of radionuclide bronchography.

Studies on the RE system and kidney function assessment in kidney transplantation will be continued on a collaborative basis between Doctors Kitani, Hayes and Moore.

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.:
06-03-01

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	2 3/4	3	3
(b) Other Tech.	1/2	1/4	1/4
Total:	3 1/4	3 1/4	3 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 31,800	$ 37,800	$ 40,100
(b) Materials & Services	7,600	7,500	8,500
(c) Indirect Expenses *	17,000	24,900	25,700
Total:	$ 56,400	$ 70,200	$ 74,300

* 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 1970

Poe, N.D. Distribution and Clearance of Inhaled Aerosols, Dis. Chest, 56: 264, 1969 (Nov.)

Taplin, G.V., Poe, N.D., Dore, E.K., Swanson, L.A., Isawa, T., and Greenberg, A. Scintiscanning and Roentgenographic Procedures in Managing Major Pulmonary Disorders, Medical Radioisotope Scintigraphy, Proceedings of the Symposium held by the International Atomic Energy Agency in Salzburg, August 6-15, 1968. Vol. II, 111-124, July, 1969.

Simmons, D.H., Levy, S.E., Olsen, C.R., Poe, N.D., Shapiro, B.J., Wasserman, K., and Wilson, A.F. Evaluation of Lung Function, Annals of Int. Med. (UCLA Conference) 71: 155-176, July 1969.

Taplin, G.V., Dore, E.K., Poe, N.D., Swanson, L.A., and Greenberg, A. Pulmonary Arterial Perfusion and Aerated Space Assessment by Scintiscanning, Frontiers of Pulmonary Radiology, Harvard Univ. Symp. April, 1967, Grune & Stratton, 2:33-75, Oct. 1969.

Poe, N.D., Bennett, L.R. Liver Scanning, J. of Geriatrics (In Press).

Poe, N.D. Lung Scanning, Humidification and Mist Therapy, International Anesthesiology Clinics, Little, Brown and Co., (In Press).

12. SCOPE OF THE PROJECT

With incorporation into the UCLA Medical Center, the activities of this section will be greatly expanded. There will be more opportunity for radioisotope studies in patients and cooperative studies with other investigators in the Medical Center. These collaborative efforts prove mutually beneficial by exchanging the technical and professional skills of this section for those of others which reduces the necessity of developing certain time-consuming technical procedures and acquiring additional expensive instrumentation. At present there are a number of active projects being carried out with the Department of Radiology and Physiology. This section will continue to serve as a basic research testing facility where new radiodiagnostic test agents can be evaluated prior to human use and where radioisotope procedures can be used to study fundamental physiologic and pathologic problems. In addition to a complete array of radioisotope detection and monitoring devices, extensive facilities for radiographic and cardiopulmonary function testing are available to carry out this aim.

For the past several years the major endeavors of this section have been directed towards defining and understanding the variables which influence the distribution of radioactive indicators in lung perfusion and inhalation scans. As perfusion scanning with albumin macroaggregates has now become widely accepted and investigated, more emphasis will be placed on radioaerosol inhalation scanning. Serial scanning is a unique method for studying the distribution and clearance of aerosols, particularly wet aerosols (which are in extensive clinical use). Efforts will be made to secure more homogeneous and reproducible aerosol generation and to establish the relationship of peripheral (alveolar) deposition to ventilation.

Other investigations will include renal studies with chelated compounds, determination of regional coronary and intestinal blood flows with labeled particles and the potential treatment of lymphogenous involvement of the liver with radiocolloids.

13. RELATIONSHIP TO OTHER PROJECTS

Related works with radioaerosols is being carried out by Doctor T. Isawa at Harbor General Hospital, Doctor K. Moser of the University of California at San Diego, Doctor Pircher in Houston, and elsewhere. Myocardial scanning in humans with albumin macroaggregates is being used in Japan by Dr. M. Endo. The basic concept of radiocolloid therapy of Hodgkin's disease of the liver was described by Doctor H. Kaplan at Stanford. Tantalum bronchography is an innovation of Doctor J.Nadel in San Francisco. Basic experiments with perfusion lung scanning are performed throughout the world but our work has been fairly closely related to that done by Doctor L. Bryant in Kentucky.

14. TECHNICAL PROGRESS IN FY 1970

Radioaerosol Deposition in Experimental Vascular Obstruction. Previous reports from this Laboratory have shown persistence of reasonably normal ventilation or aerosol deposition in lung made ischemic by experimental embolism. However, when total pulmonary artery occlusion is obtained by surgical section of the vessel, ventilation at one week is reduced by an average of 75%. This change was confirmed by Xenon gas determinations. The possibility is raised that the degree of hypoventilation is related to the degree of ischemia. This limits the use of combined perfusion-ventilation scanning in the differential diagnosis of pulmonary embolism.

Potential Use of Albumin Macroaggregates for Myocardial Scanning. The potential hazards of particulate injection into the coronary arteries are basically dose related. Therefore, it should be possible to gauge approaching toxic or excessive dosage by suitable monitoring technics and terminate particle administration. The simplest external measurements are blood pressure, heart rate and EKG. These parameters were measured in open chest animals during particle administration into the coronary vessels. Direct coronary flow & myocardial contractility measurements were also made. Very marked changes in flow and/or contractility developed before the external measurements were altered. Other approaches will have to be explored before this technic can be used clinically. However, it does appear possible to inject sufficient material for scanning without altering flow or contractility.

Tantalum Bronchography. Interesting preliminary data have demonstrated the feasibility of using tantalum dust inhalation for bronchographic studies. This material is highly inert and with the particle sizes available supposedly is rapidly and completely cleared from the lung. As this would be a desirable procedure to use in conjunction with aerosol inhalation scanning, careful clearance measurements were made following inhalation in dogs of neutron activated, commercially available particles. Our studies show a significant long-term retention of this material limiting, for the immediate future at least, the clinical use of this procedure. Further work is anticipated after varying particle size and configuration.

15. EXPECTED RESULTS FOR FY 1971

Many of the results anticipated for FY 1970 were not obtained due to the delays in the unification of laboratory facilities of this section with the Medical Center and in receipt of a new scintillation camera. As these problems have now been solved, completion of several of the deferred projects will be goals of this year.

Radioaerosol Deposition and Clearance. Particular emphasis will be placed on establishing standard and reproducible radioaerosol administration technics in humans. This is basic to the development of radioaerosol methods for diagnostic purposes (e.g., classification of chronic lung diseases, determining the distribution of medicinal aerosols, evaluating postural drainage procedures, etc.,) and is also necessary for correlation of peripheral aerosol deposition with known ventilation tests. It is postulated that aerosol deposition in the alveolar regions is a function of ventilation but this concept needs further proof.

All work will be done with wet aerosols, produced by ultrasonic generators. As particle sizing of these aerosols is practically impossible, especially after reaching the subject, efforts will be made to show that scanning with quantitation of deposition is an accurate means of estimating physical characteristics of wet aerosols and is a suitable method for comparing aerosol generators and administration technics. Work will be done in both humans and experimental animals.

Effects of Blood Gas Changes on Perfusion of Atelectatic Lungs. In continuation of studies to determine the variables influencing perfusion changes, which in turn are reflected in lung scans, further work will be done to establish the effects of contralateral pressure, pH, P_{CO_2} and P_{O_2} on flow through the acutely and chronically hypoxic lung. Previous work has demonstrated pronounced effects at different stages of atelectasis but changes in cardiac output were not considered and its effect must be clarified.

Ventilation Changes in Ischemic Lung: Effects of Pressure Breathing on Distribution of Inhaled Radioaerosols and Gases. Recent studies from this section suggest that the degree of ischemia may be a major factor in determining the magnitude of the ventilation decrease in pulmonary arterial obstruction. Acutely a decrease can be reversed by CO_2 administration but the effects of radioaerosol and gas administration at varying pressures have not been described and are important to both the understanding of basic mechanisms and the application of ventilation technics to the differential diagnosis of pulmonary embolism clinically. This work will initially be carried out in animals with simultaneous blood gas analyses to control and eliminate the variables.

Previously Initiated Studies. These studies begun in FY 1970 will be extended. First, is an attempt to further establish the safety of intra-coronary arterial administration of radioalbumin macroaggregates for myocardial scanning. Second, will be comparisons of radiogold colloid distribution in the liver after intra-arterial and intraportal injections for treatment of hepatic lymphomas. Finally, using radioactive tantalum particles, the limitations of tantalum bronchography will be further explored.

16. EXPECTED RESULTS OF FY 1972

The basic orientation of this section will be the use of radioactive tracers in the cardiopulmonary field. It is anticipated that radioaerosol research, in particular, will continue to establish the practical applications and limitations of this technic. The cyclotron produced iodine - 123 I bound to albumin will replace technetium 99m Tc being more suitable for delayed scans with its 13 hour half-life.

An attempt will be made to find a more suitable cyclotron produced gas for dynamic lung scanning. Xenon - 133 Xe has too low an energy for high quality resolution with present camera devices and should be replaced with an agent possessing approximately 150 Kev gamma photons. Other cyclotron produced gases e.g., O_2, N2 and CO_2 will be available for physiologic and clinical studies but as a rule will not be suitable for scanning.

Myocardial Scanning. While the particle method described above offers a new approach to the evaluation of coronary blood flow, its use will be limited to highly specialized situations. Other materials, which can be injected intravenously by radioactive rubidium and cesium have been tried with minimal success. Efforts to label compounds specifically utilized by the heart will be made to develop a more practical scanning agent. Specific emphasis will be placed on short-lived oxygen and carbon containing compounds. The planned acquisition of a scintillation camera for the coronary care unit at UCLA will provide an excellent facility for these studies.

Tumor Scanning. Active efforts in the direction of exploring specific tumor localizing materials suitable for scanning will be deferred to this fiscal year. Time and personnel originally planned for this activity in FY 1971 have been diverted to other tumor related studies such as lung scanning of peripheral lung nodules and intraportal injections of radiogold for treatment of hepatic lymphomas. This field is highly important and deserves intensive exploration.

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.: 06-03-01

4. Date Prepared: April - 1970

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

6. Working Location: UCLA

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

8. Project Term: From: 1963 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
Total:	2 $\frac{1}{4}$	2 $\frac{1}{4}$	2 $\frac{1}{4}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 27,900	$ 29,000	$ 29,100
(b) Materials & Services	6,000	4,400	4,400
(c) Indirect Expenses *	17,000	16,600	17,200
Total:	$ 50,900	$ 50,000	$ 50,700

* 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 IN FY 1970

Kennady, J.C., Iconoscopic Assessment of Meningiomas, Gliomas and Metastatic Brain Tumors, Proceedings of the International Symposium on Cerebral Blood Flow, Univ. of Mainz, Mainz, Germany, April, 1969, Publishers, Springer-Verlag, 1969. Cerebral Blood Flow, 5: 194-197, Sept. 1969.

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Kennady, J.C. Evaluation of Cerebral Vascular Disease, Cerebral Blood Flow, ed., Brock, Fieschi, Ingvar, Lassen, Schurmann, publishers, Springer-Verlag, Berlin-Heidelberg, New York, 3:139-143, Sept. 1969.

Carton, C.A., Kennady, J.C., Blinderman, E.E., Kobayashi,T. Jr., Cagungun, J., and Pineda, T. Extracranial-to-Intracranial Non-Suture Arterial By-Pass Grafting: Laboratory and Clinical Studies. 4th International Congress of Neurological Surgery - 9th Int'l. Congress of Neurology, Sept. 20-27, 1969, N.Y. City.(Scientific Exhibit)

Ibid. American College of Surgeons, San Francisco, Cal., Oct. 1969.

Carton, C.A., Kennady, J.C., Blinderman, E.E., Kobayashi, T.Jr., Canungun, J., and Pineda, T. Extracranial to Intracranial Non Suture Arterial By-pass Grafting. Laboratory and Clinical Studies. Excerpta Medica International Congress Series No. 193, 4th Int'l. Congress of Neurological Surgery - 9th Int'l. Congress of Neurology, Sept. 20-27, 1969, N.Y. 9:89, 1969.

Kennady, J.C., and Kim, M. Radioautography of Human Brain Tumor Cells, Clinical Research, 18:145, January, 1970.

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Batzdorf, U., Knox, R.S., Pokress, S.M., and Kennady, J. C. Membrane Partitioning of the Rose-Type Chamber For The Study of Metabolic Interaction Between Difficult Cultures, J. of Stain Technology, 44: 71-74, 1969.

Kennady, J.C., Conventional Diagnostic Procedures and Pathophysiological Considerations, Proceedings of the Symposium in Nuclear Medicine - Its Current Status in Medical Practice, Cleveland, Sept. 27-30, 1967, USAEC Conf. 670958, 40-70, 1969.

Kennady, J.C., Cole, R.E., Chin, F.K., and Hayes, M. Comparative Assessment of Brain Tumors by Angiography Scanning and the Scintillation Camera, J. of Nuc. Med., 10: 349, 1969.

Kennady, J.C., Cole, R.A., Griswold, M.L.,and Knox, R. Localization of 99m Technetium Pertechnetate in Brain Tumor Cell Cultures, J. Nuc.Med. 10:349, 1969.

Kennady, J.C., Chin, F., Hayes, M. Brain Assessment Image Intensifier Videocamera II, American Assoc. Neurol. Surgeons, April, 1970, Washington, D.C., (Scientific Exhibit).

12. SCOPE OF THE PROJECT

The objective of this section is to develop improved tracer techniques for the detection and evaluation of abnormal hemodynamics, particularly of the brain, to the level of the microcirculation. Externally performed scintillation counting and iconoscopic methods have the distinct advantage of simplicity, safety and of testing under physiological conditions.

The research activities of this section during this past year have been advancements in the qualitative and quantitative assessment of the cerebral microvasculature. This has been accomplished by a rapid bolus intracarotic injection of 99m Tc labeled albumin microaggregates (1-8μ size) and 99m Tc pertechnetate and iconoscopic recording of the test agent's passage through the cerebral hemisphere. The videoquantifier has permitted regional quantitative assessment of the test agent concentration. Hence from videotape replay of the tracer passage, polaroid pictures of the whole hemisphere and rate removal curves from any given area can be correlated with the patient's neurological status.

This section is also continuing 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 at Wadsworth Veterans Administration Hospital in Los Angeles by Doctor Oldendorf; at the University of Connecticut by Doctor Owens; in England by Doctors Harper, Jennett and Symon; in Sweden and Denmark by Doctors Ingvar, Hoedt Rasmussen and Lassen.

Studies in brain tumor culture and implantation using radioisotopes are being conducted at the University of California in San Francisco by Doctor Wilson; at Duke University by Dr. Mahaley; in Canada by Doctors Morley and Tator.

14. TECHNICAL PROGRESS IN FY 1970

Iconoscopic Studies: During this period the videotape records of the patients studied using the image intensifier videocamera have been quantified. Standardization of the videoquantifier using a 57 Cobalt source to maintain uniform instrument performance for each patient has been successful. Rate removal curves from 1.5 cm^2 regions of the cerebral hemisphere show the appearance, peak concentration and disappearance time for the test agent. This brain mapping is being done to compare the hemodynamics in each region with the overall mean values and to permit assessment of similar regions in a group of patients.

The videocamera studies correlate well with the carotid angiogram in patients with quite vascular tumors. In those patients with neurological signs of acute or chronic vascular disease, not well demonstrated by angiography, the videocamera results correlate well with the region implicated by the neurological deficit.

Human Brain Tumor Studies: Work has continued using autoradiographic techniques on human brain tumor cells cultivated on glass cover slips. Several modifications of the technique have been made this year. Results indicate that some cells in the connective tissue stroma of the tumor cultures studied actively take up the radioisotope, along with the specific tumor cells. Whether these are altered or normal connective tissue cells is being determined.

15. EXPECTED RESULTS IN FY 1971

Cerebral Hemisphere Hemodynamics: The clinical program for studying the cerebral microvasculature and regional blood volumes in patients with brain pathology using iconoscopic techniques should be continued to establish criteria for the diagnosis of specific small lesions. Improvements in resolution should be forthcoming. Continued correlations with EEG, serial angiography and histopathology is necessary. Correlation of the clinical neurological status with the videocamera changes is of prime importance.

Videocamera assessment of the effect of potential vasodilating drugs on the cerebral microvasculature will be in progress.

Calculations arising from regional brain mapping quantification will be computerized for rapid analysis.

Human Brain Tumor Culture: Studies will continue along the above described line with modifications as indicated.

16. EXPECTED RESULTS IN FY 1972

The studies outlined for FY 1971 will be continued in FY 1972. Various aspects of each problem as they arise will be pursued to a reasonable understanding and solution.

The claims regarding specific cerebral vasodilating agents will be tested objectively.

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:
06-03-01	April - 1970
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 1970	FY 1971	FY 1972
(a) Scientific	4 1/2	2 3/4	2 3/4
(b) Other Tech.	1/4	1/4	1/4
Total:	4 3/4	3	3

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 57,600	$ 37,800	$ 37,900
(b) Materials & Services	6,200	4,400	4,900
(c) Indirect Expenses *	33,900	24,900	25,700
Total:	$ 97,700	$ 67,100	$ 68,500

* 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 1970

Figueroa, W.G., and MacDonald, N.S. 54 Mn Absorption and Retention In Man Using The Total Body Counter, J. Nuc. Med. 10:402, 1969.

12. SCOPE OF THE PROJECT

The purpose of this project is to exploit the unique properties of radioactive nuclides as tracers for elucidating basic physiologic processes in health and disease states; and as clinical diagnostic aids. Specifically, the objectives embrace - : study of 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 by using radioactive forms of these materials; study of the metabolism, tissue distribution and excretory patterns of new radioactive pharmaceutical agents being evaluated for clinical usage in nuclear medicine; and finally, investigation of the metabolism of various radionuclides which are either currently or potentially significant contaminants of the human environment.

This work is carried on by means of experiments with laboratory animals and by collaborative investigations with staff physicians at the Hospital of the UCLA Center for Health Sciences.

A Total Body Counter Facility is maintained in the Medical Center for the detection and identification of extremely small quantities of radioactive materials in living human beings. It is used to measure the gamma radioactivity in children and adults drawn from the local population, in order to monitor the level of environmental contamination. In addition, it is a valuable tool in certain clinical research investigations and diagnostic tests wherein materials labeled with infinitesmal amounts of radioactive tracers are administered to patients in order to measure the absorption and retention of these materials in their bodies.

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 1970

The rates of turnover of two types of serum immune globulins were determined in 27 human subjects, using total body counting, plus blood assay following injection of the proteins labeled with Iodine-131 and Iodine-125. The data indicated that in patients with rheumatoid arthritis the rates of synthesis of immune globulin G as well as immune globulin M are about three times greater than normal. However, in another rheumatic type disease (Systemic Lupus Erythematosus) only the synthetic rate of immune globulin G is increased, with the IgM rate remaining normal. These studies, conducted in collaboration with Doctor Joshua Levy and others of the Department of Medicine, promise to be of value in clarifying the causes of rheumatic diseases and may provide a method for the objective evaluation of treatment.

The total body counter was also utilized in measuring the absorption of iron, manganese and copper in patients with hemachromatosis (iron-storage disease) following oral administration of these ions, labeled with Iron-59, Manganese-54 and Copper-67. This was a continuation of studies begun in FY 1969, in collaboration with Doctor William Figueroa, of the Department of Medicine. No significant correlation has been noted as yet between the absorption and retention of Manganese or Copper and abnormal iron metabolism.

The results of 1052 measurements of radioactive Cesium-137 made during 1960 through 1969 in 454 persons living in Southern California were summarized. These body burdens of Cs-137 arising from contamination of foodstuffs with air-borne debris from nuclear detonations reached a maximum in 1964-5 and have decreased steadily since then. From these data the total internal radiation dose from Cs-137 delivered to individual members of the population during this decade was calculated to be about 7 millirads to the total body and marrow and 4 mrad to the gonads. This is a small fraction of the radiation received over the ten year period from the radioactive Potassium-40 naturally present in the human body (150-190 mrad). A study was made of the transfer to the fetus of long-lived Tin-113 contamination usually present in Indium-113m solutions used for placental scanning of pregnant women in a growing number of hospitals. Using pregnant rabbits, it was shown that up to 3% of the radioactive tin injected into the mother does enter the fetal body. The extra radiation dose to a human fetus from this tin contamination would range from 1/10 to 1/100th the dosage from one mCi of short-lived Indium-113m injected for a placental scan (which is estimated to be under 8 millirad). A simple method to remove even this small amount of radiotin was devised.

15. EXPECTED RESULTS IN FY 1971

The Total Body Counter will continue to be used to monitor the quantities of Cesium-137 in members of the local population and to search for other radionuclides in persons exposed to radioactive material in their work, such as radiochemists, cyclotron and nuclear reactor personnel. Clinical investigations in collaboration with staff members of the School of Medicine, will include continued studies on the metabolism of labeled immune serum globulins and other blood proteins in rheumatoid arthritis and related diseases, and metabolic studies of iron, manganese and calcium. New radioactive agents for diagnostic procedures in nuclear medicine suggested by other workers in the Laboratory and Medical Center will be tested in animals to evaluate toxicity, tissue distribution, routes and rates of excretion, and other features of their metabolic behavior.

16. EXPECTED RESULTS IN FY 1972

The same types of research activity will be continued - namely, studies of the metabolic behavior of various radioactively labeled substances normally present in the body, and of labeled chemical agents administered to humans for diagnostic purposes. Production of a number of short-lived radionuclides by the Biomedical Cyclotron should be underway by 1971. 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.:	4. Date Prepared:
06-03-01	April - 1970

5. Method of Reporting:	6. Working Location:
Publications, UCLA Reports Semi-annual and Final Reports	UCLA

7. Person in Charge:	8. Project Term:
George V. Taplin, M.D. Norman S. MacDonald	From: 1967 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2 3/4	2 3/4	2 3/4
(b) Other Tech.	1/4	1/4	1/4
Total:	3	3	3

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 45,500	$ 46,600	$ 47,900
(b) Materials & Services	6,300	5,100	6,100
(c) Indirect Expenses *	25,400	33,200	25,700
Total:	$ 77,200	$ 84,900	$ 79,700

* 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 1970

Rodriguez, J., MacDonald, N.S., and Taplin, G.V. Preparation of ^{113m}Indium Albumin Aggregates for Lung and Liver Scanning, J. Nuc. Med. 10:368, 1969.

Yamada, H., MacDonald, N.S., and Taplin, G.V. Factors in Improving Yield and Quality of Technetium 99m Labeled Serum Albumin, J. Nuc. Med. 10:454, 1969.

12. SCOPE OF THE PROJECT

The function of this project is to devise new radioactive pharmaceutical agents and to improve existing agents for use in basic and clinical research in nuclear medicine. This activity entails the planning and development of appropriate chemical methods of preparation; confirming both the chemical and the radioactive identity and purity of the product; preparation of the material in sterile, pyrogen-free form suitable for administration to humans, via parenteral injection, oral ingestion or inhalation; and the design and assembly of equipment for reliable and safe preparation of these radiopharmaceuticals, on a laboratory scale, for their evaluation in animals and clinical testing by other investigative groups of the Laboratory.

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, Germany (Scheer); Mallinckrodt Institute, Saint Louis (Potchen).

14. TECHNICAL PROGRESS IN FY 1970

Improvements in the method of labeling human serum albumin with Technetium 99m included devising a simple apparatus for routine daily preparation of the material in a completely sealed and sterile system. Contamination by airborne bacteria or viruses can be completely avoided. Yields are regularly over 80% and frequently over 90%. A relatively rapid method of concentrating solutions of Tc-labeled albumin required for bolus-type injections of the order of 10 mCi per ml was found. A small, commercially manufactured pressure ultrafiltration cell is used, together with a small can of Freon gas under pressure. The filtration membrane is completely permeable to water and solutes with molecular weights below 20,000.

Although a method for preparing serum albumin macro and microaggregates labeled with Indium-113m for lung perfusion and liver scans was worked out in FY 1969, there was little demand for these preparations during FY 1970 so no refinements were made. However, a simple recipe for neutral mixtures of In-113m and albumin was used frequently for aerosol inhalation scanning of the lungs by Doctor Isawa at Harbor Hospital. In connection with these lung scanning studies, a stainless steel, gas dissolution and dispensing vessel for Xenon-133 was constructed. This device, a modification of a similar unit originated by Doctor M. Loken (University of Minnesota) provides a sterile saline solution of up to one Curie of Xe-133 obtained from Oak Ridge in a glass ampule. Portions of this solution can easily be withdrawn for use in either perfusion or inhalation lung scanning.

A solvent extraction method for separating Indium-111 from silver foil cyclotron targets is under development. This radionuclide has potential usefulness in scanning procedures which require a longer waiting time than is afforded by the 100 minute Indium-113m isotope. The In-111 should be relatively easy to produce by alpha bombardment of silver when the biomedical cyclotron is operative.

15. EXPECTED RESULTS IN FY 1971

Efforts will continue on the labeling of various chemical agents with commercially available short-lived radionuclides, notably Technetium 99m and Indium-113m, for evaluation as clinical radiodiagnostic agents by other investigators in this Laboratory, the UCLA Medical Center and affiliated institutions.

In anticipation of the production of Fluorine-18 by the biomedical cyclotron, suitable equipment will be assembled and tested to process and dispense the "raw" isotope into individual portions of solution suitable for intravenous injection into humans for bone scanning procedures. The level of activity which must be handled will be in the range of 200-500 millicuries per run. In addition, preliminary experiments will be conducted to test the feasibility of labeling certain simple organic molecules with Fluorine-18 by recoil or "hot atom" methods. The goal would be to obtain specific compounds of physiologic significance labeled with the short-lived gamma emitting F-18 for kinetic studies and/or organ scanning procedures in patients.

Chemical techniques will be worked out for separating desired radionuclides from their target matrices after irradiation in the cyclotron and converting them into appropriate pharmaceutical preparations. For example, the preparation of Gallium-67 (under investigation as a radiopharmaceutical agent for tumor localization) and Indium-111 (of potential value in cerebrospinal scanning) with the cyclotron will be evaluated. Work of this type will be closely correlated with the radiochemist on the cyclotron staff.

16. EXPECTED RESULTS IN FY 1972

The major activity during this period will be to develop means for converting the "raw" radionuclides obtained from the cyclotron operations into radiopharmaceutical agents suitable for administration to humans for use in the clinical research program in nuclear medicine. Iodine-123 and Iron 52 will receive special attention because they can be utilized immediately in currently active clinical programs, both in their simple ionic forms and in a variety of well-known, labeled compounds.

Effort will also be directed to the labeling of various, simple, organic compounds such as amino acids and monosaccharides, with the short-lived Carbon-11 which should be available at this time. Such materials will be readily detectible by external scintillation detectors (such as scanners and cameras) soon after administration, and may provide valuable diagnostic information about their distribution and catabolism in illnesses and metabolic imbalances.

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.:
06-03-01

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA

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

8. Project Term:
From: 1967 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	2 1/4	2	2
(b) Other Tech.	3/4	1/4	1/4
Total:	3	2 1/4	2 1/4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 41,100	$ 25,700	$ 25,800
(b) Materials & Services	7,500	7,400	8,400
(c) Indirect Expenses *	25,400	16,600	17,200
Total:	$ 74,000	$ 49,700	$ 51,400

* 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 1970

Webber, Milo M., Bennett, L. R., Cragin, Michael, and Webb, Roscoe Jr., Thrombophlebitis--Demonstration by Scintiscanning, Radiology 92:3:620-623, 1969.

Cragin, Michael D., Webber, Milo M., Victery, Winona, and Pintauro, Daniel, Technique for the Rapid Preparation of Lung Scan Particles Using 99m-Technetium-Sulfur and Human Serum Albumin, J. Nucl. Med. 10:10:621-623, 1969.

Larson, Jeanne M. and Bennett, Leslie R., Human Serum Albumin as a Stabilizer for 99m-Tc-Sulfur Suspension, J. Nucl. Med. 10:6:294, 1969.

Gyepes, Michael T., Bennett, L. R., and Hassakis, Patricia C., Regional Pulmonary Blood Flow in Cystic Fibrosis, Am. J. Roent., Rad. Ther. & Nucl. Med. 106:567, 1969.

Surprenant, E., Wilson, A., and Bennett, L. R., Clinical Application of Regional Pulmonary-Function Studies, J. Nucl. Med. 10:6:374, 1969 (abstract).

Webber, Milo M., Bennett, L. R., Cragin, Michael, Victery, Winona, and Webb, Roscoe Jr., Thromboscintigraphy--Thrombosis Demonstrated by Scintiscanning, J. Nucl. Med. 10:6:379, 1969 (abstract).

Webber, Milo M., Normal Gamma Radiation Imaged Brain Examination Including Variation in the Normal, to be published in Radiology of the Skull and Brain, Thomas H. Newton, ed., (in press).

Webber, Milo M., participating author, Liver and Pancreatic Scanning, edited by Clark, UCLA Interdepartmental Clinical Case Conference, California Medicine (in press).

Poe, N. D. and Bennett, L. R., Liver Scanning, Geriatrics, (in press).

Bennett, Leslie R., Diagnosis of Cardiovascular Disease, to be published in Nuclear Medicine, William H. Blahd, ed., Second Edition, McGraw-Hill Book Co., Inc., (in press).

12. SCOPE OF THE PROJECT

This project has been established to study the clinical applications of short-lived isotopes. The advent of such short-lived isotopes as Tc^{99m} has revolutionized the field of radioisotope scintigraphy. This project is designed to develop and refine clinical methods in order to obtain the greatest clinical utilization of such short-lived isotopes. Characterization and refinement of now commonly used tracer materials such as Technetium-sulfur suspension are examples of the type of work that is being done. The short-lived isotope Fluorine-18 has been extensively studied for its possible use in bone scanning. A long-term project is now underway in which Fluorine-18 rather than longer-lived tracers is being used in order to evaluate its practical applicability. There is great potential advantage in a short-lived tracer for bone scanning purposes which has proven to be superior to radiographic methods in many instances where bony metastases are expected. The use of antibodies specifically for organs is another subject of investigation of this project. Such studies have begun. The applications are numerous and have promising possibilities.

The detection of intravascular fibrin deposits such as are found in thrombophlebitis has been demonstrated. The mechanism and possible clinical applications of such localizations are to be further refined and developed. Comparison of this method to that of antifibrin, as described by Bale and Associates, will be made.

Very short-lived radioactive gases, such as are produced in the cyclotron, will eventually be available, and a study of their characteristics and use in clinical applications is also within the scope of this project. The use of Xenon gas, which is radioactive, in the evaluation of ventilation of various portions of the lung is an active part of the project.

13. RELATIONSHIP TO OTHER PROJECTS

This project is related to the radiodiagnostic agent development sections and to those of the basic nuclear medicine section. Short-lived Technetium compounds are being investigated in many laboratories throughout the United States including Brookhaven National Laboratory, Johns Hopkins University, and the University of Chicago. Antifibrin antibody studies at the University of Rochester were initially performed by Dr. William Bale. Fluorine-18 compounds initially were developed at the Roswell Park Memorial Hospital by Drs. Blau and Bender.

14. TECHNICAL PROGRESS IN FY 1970

Technetium compounds in the form of stabilized Technetium-Sulfur colloid have been extensively used as a bone marrow scanning agent in order to demonstrate blood flow to the femoral head. Numerous patients who have undergone routine scanning of the liver have also had the femoral head area examined. The normal pattern in these patients appears to be symmetrical. Fluorine-18 has proved to be a satisfactory agent for bone scanning; patients can be scanned over extensive areas in a short time, and satisfactory demonstration of metastatic lesions of bone is possible. Indium compounds have been introduced as a substitute for previous use of technetium labeled albumin in blood pool scanning to demonstrate cardiac blood pool and the placenta.

Continuing studies are underway on localization of intravascular clot formation. Cine photomicroscopy has shown the dynamics of technetium labeled macroaggregates of albumin attraction to clots in *in vitro* systems.

We are searching for a suitable *in vitro* system for comparison of various particles in clot localization studies. It has been found that various particulate radiotracers other than macroaggregates of albumin do attach to clots *in vivo* and *in vitro*. Xenon gas ventilation and perfusion studies of the lung in asthma, cystic fibrosis, and other chronic lung diseases have been investigated as to their clinical usefulness.

15. EXPECTED RESULTS IN FY 1971

Continued studies of indium and technetium compounds with especial emphasis upon dynamic studies of cerebral and cardiac circulation in clinical situations will be instituted. The evaluation of dynamic "flow studies" done in conjunction with routine brain scanning will be carried out in this year. Tagged antibody studies will be continued to determine their suitability as agents for demonstration of organs or disease processes within organs.

Studies of vascularity of the femoral head in patients with known hip disease will be performed to correlate with previously obtained normal data. Studies of cerebral spinal fluid dynamics with especial interest in demonstration of inflammatory changes of the meninges will be explored.

16. EXPECTED RESULTS IN FY 1972

Cyclotron availability by this time is expected. Studies of a dynamic type using very short-lived tracers such as O-15 and I-123 will be investigated as to their potential use. In clinical studies, ventilation patterns may prove important to clinical management, and this may be obtained more easily using the short-lived tracers available from the cyclotron than with currently available Xenon-133.

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.:
06-03-01

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA

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

8. Project Term:
From: 1969 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	$\frac{1}{2}$	$3\frac{1}{2}$	5
(b) Other Tech.	$\frac{1}{4}$	$1\frac{1}{2}$	$1\frac{1}{2}$
Total:	$\frac{3}{4}$	5	$6\frac{1}{2}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 14,800	$ 62,500	$ 84,000
(b) Materials & Services	1,800	15,600	21,100
(c) Indirect Expenses *	8,500	41,500	51,500
Total:	$ 25,100	$ 119,600	$ 156,600

* 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 1970

None

12. SCOPE OF THE PROJECT

A large number of radioactive nuclides are of very great potential value to nuclear medicine and biology because of their distinctive physical properties, such as radioactive half-life, mode of decay (e.g., electron capture, positron emission), and energy of emitted radiations. Most of these can be prepared only by the transmutation of stable atoms by interaction with energetic charged particles from an accelerator. They cannot be prepared by neutron irradiation in a nuclear reactor.

The mission of the new Biomedical Cyclotron Facility is to produce radioactive nuclides for clinical research and physiologic studies in nuclear medicine, and to provide a source of charged atomic particles of moderate energies for other biologically oriented research. The radioactive materials will be made available to investigators not only at UCLA, but also at several other hospitals in the community. Another activity of the facility will be the development and application of methods of measuring trace elements in small samples of biologic and medical interest by charged-particle activation techniques, particularly in situations where other methods are too insensitive. The beams of accelerated protons, deuterons and helium ions will also be used in collaborative investigations with other Laboratory and University programs 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) and Mt. Sinai Hospital in Miami, Florida (Gilson).

14. TECHNICAL PROGRESS IN FY 1970

Following allocation of a site for the cyclotron facility at the Center for Health Sciences and arrangement for financing construction of housing for the accelerator by the University, preliminary plans were drawn and submitted to the University Office of Architects and Engineers, who then engaged the architectural firm of Neptune and Thomas, Inc., to prepare the necessary construction documents. These were completed and approved in final form in February of 1970 and construction quotations were received from general contractors in March. The schedule calls for completion in September-October 1970.

Requests for quotations for the accelerator were answered by two manufacturers. After careful, detailed evaluation of the technical features of the machines and lengthy negotiations, a purchase order was awarded to The Cyclotron Corporation (Berkeley, California) in October, 1969. The cyclotron will probably be completed and ready for delivery well before construction of the building is finished.

Considerable effort was expended in planning details of the building in consultations with the architects and engineers. Calculations of the radiation shielding requirements were carried out and a detailed operations program for radiation safety was written for review by the Campus Safety Committee and the State of California Department of Radiological Health which will authorize operation of the accelerator. The requirements for ancillary equipment and supplies in support of the accelerator were estimated as well as was possible in advance of delivery of the machine and individual items were procured to the extent that funds permitted.

15. EXPECTED RESULTS IN FY 1971

Delivery of the cyclotron is expected in late September of 1970 and installation and acceptance tests should be completed by the end of November. "Shake-down" runs will be conducted during December so that productive operation of the accelerator should begin in early January of 1971. During the latter half of FY 1971 it is anticipated that production of Fluorine-18 for clinical research (primarily bone scanning) will become routine and development of techniques for preparing other radionuclides will be underway. The nuclides which will receive first attention are Iodine-123, Iron-52 and Carbon-11, in the forms of carbon monoxide and carbon dioxide. The mechanical devices to present the target to the bombarding beam will necessarily be different for each of these nuclides so that considerable effort will have to be devoted to these practical problems of "targetry". Advantage will be taken of the experience of others in this field wherever possible. Full assistance will be rendered by the tool designer, machinist and electronics section of the Laboratory and should be quite adequate for these needs. The chemical manipulations necessary to recover the radioactive products from the targets after irradiation will also have to be worked out for each case. The assistance of a full-time radiochemist is anticipated in July 1971 and substantial progress should be made in developing chemical techniques even before the accelerator is functional.

16. EXPECTED RESULTS IN FY 1972

During this period the routine production of Fluorine-18 will continue and Carbon-11 (dioxide and monoxide) will probably become readily available in such quantities as to satisfy all clinical research requests. Further exploitation of the biomedical potentialities of Carbon-11 will depend on the development of rapid methods for labeling and purifying organic compounds of physiologic significance with this radionuclide. It is anticipated that exploratory work during FY 1971 by other groups such as the Radiodiagnostic Agent Development Section using Carbon-14 will have provided the necessary "know-how" to apply Carbon-11 labeling to several compounds of potential value in nuclear medicine. Efforts will be made to prepare sufficient quantities of Iodine-123 and Iron-52 to supply the investigators with these nuclides either for direct usage in scanning or for labeling other agents. It is anticipated that development of the techniques for producing these two important nuclides reliably and in reasonably good yields will be completed early in FY 1972. The production of Oxygen-15 (2 minutes half life) by deuteron bombardment of nitrogen gas has been reported by others to be simple and efficient. The logistical problems of producing and transporting

this gas to the Clinical radioisotopes area of the Department of Radiology in the Medical Center for use in nuclear medicine will, hopefully, be solved by installation of a pneumatic tube delivery system during this year.

During this period collaborative studies will be undertaken which will involve the direct irradiation of purified compounds of biologic interest in programs of the Radiobiology Division of the Laboratory.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title:
Leukemia Biology

3. AEC Budget Activity No.:
06-03-01

4. Date Prepared:
April - 1970

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 1970	FY 1971	FY 1972
(a) Scientific	4	4	4
(b) Other Tech.	-	-	-
Total:	4	4	4

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 51,900	$ 55,400	$ 57,800
(b) Materials & Services	7,400	7,500	7,500
(c) Indirect Expenses *	33,900	33,200	34,300
Total:	$ 93,200	$ 96,100	$ 99,600

* 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 1970

Hays, Esther F., "The Effect of Epithelial Remnant and Whole Organ Grafts of Thymus on the Recovery of Thymectomized Irradiated Mice." J. Exp. Med. 129, 1235-1246, (1969).

Vredevoe, D.L., and Hays, E.F., "Effect of Antilymphocytic and Antithymocytic Sera on the Development of Mouse Lymphoma." Cancer Res. 29, 1685-1690, (1969).

Hays, Esther F., and Alpert, P.F., "Effects of Short-Term Epithelial Reticular Cell and Whole Organ Thymus Grafts in Neonatally Thymectomized Mice." J. Exp. Med. 130, 847-857, (1969).

Vredevoe, D.L., and Hays, E.F., "The Effect of 6-Mercaptopurine and Imuran on the Development of Mouse Lymphoma." UCLA #12-729, (1969).

Hays, E.F., "The Role of Lymphocytes and Epithelial Cells in Restoring Immunologic Capability in Thymectomized Mice." Clin. Res., 18, 179, (1970).

12. SCOPE OF THE PROJECT

The relationship of the thymus to the development of lymphoma in the mouse.

The thymus is the target organ for development of lymphoma associated with Gross virus and is the primary or sometimes the only site of lymphoma in animals dying of this disease. Removal of this organ prevents lymphoma development. When the thymectomized AKR mouse (a strain of high spontaneous lymphoma incidence, the onset of which is accelerated by administration of Gross virus to suckling mice) is grafted under the renal capsule with syngeneic neonatal thymus in the presence of virus, there is a high incidence of lymphoma developing in such grafts. It has been determined that lymphoma, in this system, is a result of direct malignant transformation of thymus lymphocytes under the influence of lymphomagenic virus and thymus epithelial reticular cells. Studies have been designed to take a closer look at factors affecting lymphomagenesis in this system such as: the time of virus exposure to the thymus used for grafting; the age of the graft; and the action of irradiation and drugs with known properties of metabolic inhibition before and after exposure of the thymus graft to virus. Results obtained from these studies will increase our knowledge of events occurring in the thymus prior to lymphoma development.

Immunologic response in pre-lymphoma mice.

Since the thymus is directly involved in lymphomagenesis and also has an important role in immunologic reactions in the mouse, it would follow that in the pre-lymphoma period there could be an alteration of thymus-related immunologic function. Studies of various parameters of immune responsiveness in AKR and C3H mice given Gross virus as newborns have been compared to those in normal C3H mice. Also, the ability of spleen cells from parental strains having prior exposure to lymphomagenic virus have been studied with regard to their capability to produce graft vs. host (GVH) reactions when inoculated into suckling F_1 hybrid mice. Observations of this type are of value because they show whether or not there is functional impairment of the thymus prior to the development of lymphoma. If such impairment is found its role in the development of neoplastic transformation of thymus lymphocytes must be investigated.

Immunosuppression and the development of lymphoma.

The use of antilymphocytic and antithymocytic sera as immunosuppressive agents in patients receiving homografts have posed the questions, with some actual evidence to support them, as to whether immunosuppression would allow the appearance of neoplasms with tumor antigens that were being effectively held in check prior to this type of therapy, or whether antiserum treatment would promote the unrestrained proliferation of latent oncogenic viruses. The mouse lymphoma system would seem to be ideal for studying this, since the lymphoma is of viral etiology and the lymphoma cells have been shown to have tumor specific antigens. Heterologous antithymocytic (ATS) serum has been prepared by inoculation of rabbits with C3H mouse thymocytes and studied by its administration to virus inoculated hosts, or by incubation of lymphoma cells with antiserum prior to their inoculation. Since the lymphoma cell is thymus derived, a dual role of these antisera must be postulated. An immunosuppressive effect on the host producing a situation which would promote growth of antigeneic tumors, and a cytotoxic effect on the lymphoma cell itself. This aspect of the work is carried out under the supervision of Donna Vredevoe, PhD, a consultant to the laboratory.

13. RELATIONSHIP TO OTHER PROJECTS

The following is a list of some laboratories where research in experimental leukemia and the role of the thymus in the immune mechanism are being carried out. The work of these investigators is related to that carried out in our laboratory. Dr. L. Gross, Veterans Hospital, Bronx, New York. Dr. H.S. Kaplan, Stanford University,School of Medicine, Stanford, California. Dr. J.F.A.P Miller and Dr. Donald Metcalf, Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Melbourne, Australia. Dr. W.H. Hildemann and Dr. Marcel Baluda, Department of Medical Microbiology and Immunology, University of California, Los Angeles. Dr. C.G. Craddock, Jr., Department of Medicine, University of California, Los Angeles. Dr. David Imagawa, Department of Pediatrics, University of California, Los Angeles. Dr. William Carnes, Department of Pathology, University of California, Los Angeles.

14. TECHNICAL PROGRESS IN FY 1970.

The relationship of the thymus to the development of lymphoma in the mouse.

Studies of the microscopic morphology of grafts of thymus in AKR mice exposed to lymphomagenic virus have been completed. In this system, where all of the grafts ultimately undergo transformation to lymphoma, biopsies of the grafts in the pre-lymphoma period where found to be morphologically identical to similar grafts placed in AKR mice not exposed to virus, and which developed a low incidence of lymphoma. In both instances the grafts have the morphology of normal thymus. These studies lead to the conclusion that pre-lymphoma changes of thymus described in the literature may well be non-specific changes related to aging rather than due to any direct effect of the virus. From observations made in our studies, we believe that transformation to lymphoma results from inhibition of maturation of thymus lymphocytes which can occur only in the presence of virus and thymus epithelial reticular cells. It has also been shown that heavy irradiation with 5000r ^{60}Co to the neonatal thymus prior to grafting does not prevent regeneration of the graft (i.e. does not destroy epithelial reticular cell function) and

that when these grafts are placed in the virus infected AKR mouse, lymphoma develops in over half of the grafts. However, if these irradiated syngeneic grafts are exposed to virus in vitro or in vivo prior to grafting to AKR mice not given additional virus, lymphoma does not develop. These observations imply that irradiation inhibits initiation of viral infection as well as the progression of an established infection in the thymus, but that a heavily irradiated thymus graft can develop lymphoma when placed in a virus-infected host.

Immunologic response in pre-lymphoma mice.

These studies have been carried out in AKR mice (a strain with high lymphoma incidence), C3H mice given lymphomagenic virus at 2 to 3 days of age (ultimate lymphoma incidence 60%), and normal C3H mice (animals that rarely develop lymphoma). Animals from 1 month to 1 year have been studied. No impairment of production of immunoglobulins was found in these mice. The agglutinin and hemolysin responses to sheep red blood cells (SRBC) was identical in the three groups studied. Cellular immunity was accessed by measuring macrophage migration inhibition (a normal property of sensitized lymphocytes in the presence of antigen). The lymphocytes in the two groups of pre-lymphoma mice were found not to inhibit macrophage migration. This occurred at all ages studied, thus indicating a functional abnormality of these cells. Further evidence of functional impairment of pre-lymphoma lymphoid cells was found when the ability of spleen cells from AKR and SJL/J mice to produce a GVH reaction in AKR x SJL/J F_1 hybrid recipients was compared. The AKR cells showed a reduced ability to mount a GVH reaction. It is known that the thymus has an important role in the development of normal cellular immune reactions in the mouse, and observations such as the above, fit with the concept of an alteration of thymus function (possibly virus induced) preceding lymphoma development.

Immunosuppression and the development of lymphoma.

When heterologous antithymocytic serum (ATS) of known immunosuppressive capacity was administered to virus inoculated AKR mice in various schedules during the pre-lymphoma period the incidence of disease was unchanged. However, when similar sera were given to C3H mice inoculated with lymphomagenic virus as newborns or to normal AKR mice some decrease in lymphoma incidence compared with that of controls was found. Virus given to adult C3H mice during a course of ATS administration resulted in the development of lymphoma in a few animals. It did not develop in normal rabbit serum treated controls given virus. These studies have indicated that administration of ATS does not alter the virus-thymus interaction that results in lymphoma in AKR mice. Decrease in incidence in virus inoculated neonatal C3H and normal AKR mice may be due to an antiviral action of the serum since Gross viral antigen is ubiquitous in mice. Lymphoma occurring in adults after ATS administration with virus is probably the result of immunosuppression. Further studies have shown that when lymphoma cells are incubated with ATS prior to grafting to syngeneic hosts they are inactivated. When ATS is administered to hosts prior to inoculation of allogeneic lymphoma cells tumor growth results. These observations demonstrate two properties of these sera, that of direct inactivation (cytotoxicity) of tumor cells, and of immunosuppression of host animals promoting acceptance of tumor allografts.

15. EXPECTED RESULTS FY 1971.

It is now known that the bone marrow provides stem cells which enter the thymus and mature there to become thymus lymphocytes and which then leave the thymus to function as antigen reactive cells. Also, the bone marrow provides cells which go directly to the spleen and possibly to other lymphoid tissues to act as antibody forming cell precursors. We have demonstrated that neonatally thymectomized mice given adult bone marrow cells at birth are prevented from developing wasting disease, although their response to SRBC remains impaired. These animals have the characteristic lymphocyte depletion in spleen and nodes, but the microscopic morphology of the Peyer's patches is relatively normal. To further study the bone marrow-thymus interrelationship thymectomized irradiated adults will be protected with syngeneic and allogeneic bone marrow cells of two types, one with the same H-2 locus as the host and the other differing from the host at H-2. These animals will then be given thymus grafts from these same three strains and studied to observe (1) regeneration of the grafts (2) responsiveness to SREC and (3) microscopic morphology of the lymphoid tissue.

The effect of various inhibitors of nucleic acid synthesis on lymphomagenesis will be ascertained using the system of virus exposed thymus grafts in AKR mice. This will be done to further our understanding of the role of virus in neoplastic transformation.

The relationship of GVH reactions to lymphomagenesis which is being studied at present will be completed. Continued observations of these animals have confirmed our initial impression that a high incidence of short latent period, thymic lymphoma results when virus infected spleen cells are inoculated in a system where a GVH reaction is minimal. And in a system without lymphomagenic virus but which produces a strong GVH reaction animals that survive develop non-thymic lymphomas after a prolonged latent period. The effect of virus in this second system will be ascertained.

16. EXPECTED RESULTS FY 1972.

The following questions can be asked, hopefully to be answered with future studies. (1) Does virus act directly on the genetic mechanisms of the maturing thymocyte to cause a cessation of maturation at the lymphoblast stage? The lymphoma cell in this instance would be a thymocyte precursor which had lost its ability to mature. (2). Is transformation to lymphoma the result of a virus induced change in a thymus lymphocyte which makes it a completely new cell type? Or (3) does virus act on the epithelial cells to abolish their directive function on the maturation of the thymus lymphocyte?

The system of predictable transformation to lymphoma in thymus grafts placed under the kidney capsule presents an opportunity to study the factors involved in this neoplastic change. After completing our investigations with metabolic inhibitors, we will initiate studies to determine if the observed effects of these drugs are due to an alteration of viral replication or to a inhibition of some epithelial cell function which directs maturation of thymus lymphocytes. Clearly, it would be important to elucidate the point of virus-cell interaction which results in lymphoma.

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.:
06-03-01

4. Date Prepared:
April - 1970

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

6. Working Location:
UCLA

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

8. Project Term:
From: 1954 To: Continuing

9. Man Years	FY 1970	FY 1971	FY 1972
(a) Scientific	3	$\frac{3}{4}$	$\frac{3}{4}$
(b) Other Tech.	-	-	-
Total:	3	$\frac{3}{4}$	$\frac{3}{4}$

10. Costs	FY 1970	FY 1971	FY 1972
(a) Direct Salaries	$ 46,200	$ 18,700	$ 18,700
(b) Materials & Services	4,300	400	400
(c) Indirect Expenses *	33,900	8,300	8,600
Total:	$ 84,400	$ 27,400	$ 27,700

* 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 1970

Hennessy, T. G., Jean Kinnear, and Dagmara Davis: Dose Response - Erythropoietin Units Versus Percent Uptake of Fe^{59}. USAEC Report UCLA-12-724 (1969).

Hennessy, T. G., Jean Kinnear, and Dagmara Davis: Evaluation of Hypoxia-Induced Polycythemic Mice. USAEC Report UCLA 12-724 (1969).

Frey-Wettstein, M., C. G. Craddock, T. G. Hennessy, Jean Kinnear and Dagmara Davis: Erythropoiesis in Testosterone Treated Rats. USAEC Report UCLA 12-724 (1969).

12. SCOPE OF THE PROJECT

This project will become inactive in FY 70 to permit Dr. Hennessy to become Chief of the Radiation Protection and Scientific Services Division. As Chief of this Division Dr. Hennessy will be responsible for Health Physics activities including those related to the cyclotron project. It is to be hoped that Dr. Hennessy will have opportunities to engage in collaborative research with colleagues. However, because of the stringency of Laboratory funds and his other responsibilities no direct research support through the Laboratory is possible.

In the past this project has been concerned with 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 1970

The major effort during the current fiscal year has been devoted to the evaluation of erythropoietin as a therapeutic agent in radiation injury of bone marrow. In a series of experiments using radioactive iron as our diagnostic tool we have determined that, at the radiation level of 500 rad, erythropoietin is effective in accelerating recovery from erythropoietic bone marrow suppression. The administration of the erythropoietin must take place during the first day after the irradiation since a delay of one day makes the treatment ineffective. Also additional treatment after the

first day does not increase the effectiveness. It was also shown in accompanying lethality studies that recovery of the red cell system alone, which was induced by the erythropoietin, does not improve the survival.

Additional studies have continued during this year on a possible inhibitor of erythropoietin in plasma of patients with aplastic anemia. This inhibitor appears to be activated by normal plasma. Also additional cases of various disease states in which erythropoietin is in excess have been studied.

15. EXPECTED RESULTS IN FY 1971

Project to be discontinued. See Section 12.

16. EXPECTED RESULTS IN FY 1972

Project to be discontinued. See Section 12.