Master

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

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

RESEARCH AND DEVELOPMENT PROGRAM
FISCAL YEAR 1973

APRIL 1971

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

U. S. Atomic Energy Commission
SAN FRANCISCO OPERATIONS OFFICE

Biology and Medicine
PROGRAM

RESEARCH AND DEVELOPMENT PROGRAM

INTRODUCTORY STATEMENT

The overall objectives of the Laboratory of Nuclear Medicine and Radiation Biology may be summarized as follows:

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

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

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

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

Programmatic adjustments continue to be made in an attempt to focus on problems where we have the highest scientific capability and increasing support will be given to our strong research programs in Nuclear Medicine, Biochemistry, and Developmental Cell Biology.

FY 1972 will be the first full year of operation for the Biomedical Cyclotron. To further capitalize on this important research capability, we will expand research in radiopharmacology through the addition of a new staff member.

A small but very strong program has been assembled for Developmental Cell Biology investigations. The addition of Dr. Gerschenson to our staff gives us four very able investigators in this area. This new group will have a high degree of interaction with our Biochemistry and Radiation Biology Divisions, and will also provide a certain amount of support for the Nuclear Medicine and Cyclotron activities.

The program on late effects of radiation is being phased out in favor of a modest program on radiation effects at the organismal level.

The most urgent Laboratory problem is for recruitment in Radioecology. A vigorous search is underway for an experienced ecologist. After an initial appointment is made, a second and third would follow. One of the two latter appointments would be using an FTE provided by the Chancellor for a joint appointment with Botany.

Under consideration at present is the establishment of an expanded program in Instrumentation which would complement work in Nuclear Medicine and capitalize on the presence of the Cyclotron, as well as provide support for such areas as Radioecology and Radiation Biology. We would visualize a nucleus of five scientists in this area, but a significant amount of additional support would be necessary to develop a viable program. Specific proposals will be developed in the course of recruitment negotiations with Mr. Gerald Huth and will be coordinated with the Division of Biology and Medicine prior to proceeding.

The budget we are requesting for FY 1972 represents a $12,000 increase over the Congressional Budget, but is still down $71,000 from FY 1971. The head-count next year will again be reduced, bringing Laboratory employment down to 65% of the level of FY 1965 when the current budgetary difficulties began. Merit increases and probable range adjustments for some of the staff will place additional stresses on the budget. It should also be kept in mind that about 13% of our current budget goes for items over which we have no control, such as rent, management allowance, and utilities. To meet next years budget request, several programs are being eliminated and others are adjusted downward. Support functions such as secretarial staff, accounting, library, shops and vivarium are at irreducible minima.

COST INFORMATION:

The composition of costs and staffing for FY 1971, FY 1972, and FY 1973, are summarized below for the entire Biology and Medicine Program by major categories of expense.

	FY 1971	FY 1972	FY 1973
I. Costs: (Shown in Thousands)			
Salaries (Direct)	$1,217.7	$1,176.0	$1,248.0
Supplies, Travel, & General Expense	347.3	328.0	377.3
Indirect Costs	830.0	820.0	850.0
Total Operating Costs	$2,395.0	$2,324.0	$2,475.3
II. Manpower: (Shown as Full Time Equivalence)			
Direct Man Years	98	92	95 $\frac{1}{2}$
Direct Scientific Man Years	87 $\frac{1}{4}$	80 $\frac{1}{2}$	83 $\frac{1}{4}$
III. Cost Per Man Year Data: (Shown in Thousands)			
Cost Per Direct Man Year	$ 24.5	$ 25.2	$ 26.1
Cost Per Direct Scientific Man Year	$ 27.6	$ 28.8	$ 29.7

Total Cost and Manpower data for individual research projects are summarized by Biology and Medicine Activity categories in the chart on Page iv. 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 1971	FY 1972	FY 1973
Composition of Indirect Expense:			
I. Manpower (Shown as Full Time Equivalence):			
Administrative Services	24	23	24
Technical Services	10	7	7
Building Maintenance	6	6	6
Total Indirect Personnel	40	36	37
II. Costs (Shown in Thousands)			
Administrative Services	$ 335.5	$ 331.0	$ 349.9
Technical Services	119.4	105.7	108.8
Building Maintenance	65.1	68.3	71.5
Building Amortization	126.7	126.7	126.7
Utilities	60.1	63.1	65.1
U. C. Management Allowance	75.0	75.0	75.0
U. C. Accounting and Purchasing Services	36.0	37.0	38.0
Miscellaneous (Laundry, Postage, General Supplies, etc.)	12.2	13.2	15.0
Total Indirect Costs	$ 830.0	$ 820.0	$ 850.0

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 1971 COSTS	FY 1971 MAN YRS	FY 1972 COSTS	FY 1972 MAN YRS	FY 1973 COSTS	FY 1973 MAN YRS
06 01 01	Effects of Radiation on Living Organisms						
	Medical Physics Problems (Cassen)	$ 36.9	1 3/4	$ -0-		$ -0-	
	Applied Mammalian Radiobiology (Byfield and Bennett)	28.1	2	25.7	1 1/4	29.5	1 1/4
	ACTIVITY TOTAL	$ 65.0	3 3/4	$ 25.7	1 1/4	$ 29.5	1 1/4
06 01 02	Molecular and Cellular Radiobiology						
	Macromolecular Chemistry (Simmons)	$ 18.2	3/4	$ 4.0	0	$ 4.2	0
	General Metabolism (Mead)	89.5	3 1/2	72.9	3	77.6	3
	Organic Chemistry (Howton)	71.7	2 1/2	73.0	2 1/2	76.7	2 1/2
	Tracer Synthesis (Nevenzel)	30.2	1 3/4	43.2	2	45.5	2
	Biosynthetic Control (Fulco)	51.8	2 1/2	60.8	2 1/2	64.0	2 1/2
	Developmental Regulation (Harary)	107.2	4 1/2	116.4	5	121.6	5
	Developmental Neurobiology (Herschman)	59.3	2 1/2	67.0	2 1/2	70.7	2 1/2
	Mammalian Cell Biology (Gerschenson)	-0-	0	34.1	1 1/2	36.1	1 1/2
	Developmental Radiobiology (de Vellis)	73.8	3	71.9	3	77.3	3
	Chemical Radiobiology (Myers)	118.7	4 1/2	101.7	3 1/2	110.1	4 1/2
	Sub-Cellular Radiobiology (Ward)	59.0	3	90.9	4	93.9	4
	Physical Radiobiology (Strickland)	69.6	3 1/2	86.4	4	91.6	4
	ACTIVITY TOTAL	$ 749.0	32	$ 822.3	33 1/2	$ 869.3	34 1/2
06 02 04	Land and Fresh Water Environmental Sciences						
	Soil Factors (Nishita)	$ 90.2	3	$ 89.8	3	$ 95.3	3
	Plant Factors (Romney)	92.9	3 1/4	87.8	3	95.8	3 1/4
	Environmental Factors (Hawthorne)	74.5	3 1/4	71.5	3	77.9	3 1/4
	Radiation Ecology (Maza-Acting)	60.7	2 1/4	49.9	2	91.6	3 1/4
	Ecology of the Nevada Test Site (Beatley)	69.7	3 1/4	61.3	2 1/2	54.8	2 3/4

	Radioecology (Turner)		71.8	2		84.0	3		90.9	3 ½
	Chemical Problems (Wood)		121.0	5 ½		115.5	5 ½		101.6	5
	Physiology of Mineral Accumulation in Plants (Lunt)		29.2	1		29.3	1		45.0	1 ½
	Quantitative Plant Ecology (Wallace-Acting)		67.6	2 ¾		61.1	2 ½		66.0	2 ¾
	Plant Physiology Ecology (Wallace)		75.5	3		53.8	2		73.0	2 ½
	Soil Survey and Characterization (Hale)		31.0	1 ¼		28.4	1		-0-	0
	Functional Morphology and Relationships to Ecology (Wallace)		-0-	0		-0-	0		32.9	¾
	Distribution and Interrelationship of Elements in Biological Systems (Alexander)		29.9	1		25.1	1		25.6	1
	ACTIVITY TOTAL	$	814.0	31 ½	$	757.5	29 ½	$	850.4	32 ½
06 02 08	Radiological & Health Physics & Instrumentation									
	Medical Physics Instrumentation (Cassen)	$	47.0	2 ½	$	46.4	2 ¼	$	47.0	2
	ACTIVITY TOTAL	$	47.0	2 ½	$	46.4	2 ¼	$	47.0	2
06 03 01	Cancer and Other Clinical Research									
	Clinical Nuclear Medicine (Taplin)	$	136.5	5 ¼	$	114.0	4	$	104.7	3 ¾
	Basic Nuclear Medicine (Poe)		71.4	3 ¼		85.3	3 ¼		80.1	3 ¼
	Hemodynamics (Kennady)		58.1	2 ¼		-0-	0		-0-	0
	Nuclide Metabolism (MacDonald)		66.8	3		64.8	2 ½		65.7	2 ½
	Radiodiagnostic Agent Development (Taplin and MacDonald)		84.6	2 ¾		109.1	4		102.1	4
	Clinical Studies: Short-Lived Isotopes (Webber)		48.3	2 ¼		55.5	2 ½		58.2	2 ½
	Biomedical Cyclotron Facility (MacDonald and Cassen)		128.3	4 ¼		151.7	5 ¼		183.2	6
	Leukemia Biology (Hays and Vredevoe)		98.0	4 ½		91.7	4		85.1	3 ¼
	Mammalian Radiobiology (Hennessy)		28.0	¾		-0-	0		-0-	0
	ACTIVITY TOTAL	$	720.0	28 ¼	$	672.1	25 ½	$	679.1	25 ¼
	TOTAL 060000 PROGRAM-	$	2,395.0	98	$	2,324.0	92	$	2,475.3	95 ½

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: MEDICAL PHYSICS PROBLEMS

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

4. Date Prepared: April - 1971

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: 1971

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	1 3/4	0	0
(b) Other Tech.	-	0	0
TOTAL:	1 3/4	0	0

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 18,000	$ 0	$ 0
(b) Materials and Services	1,500	0	0
Sub-Total Direct Project Support	$ 20,300	$ 0	$ 0
(c) Indirect Expenses *	16,600	0	0
TOTALS:	$ 36,900	$ 0	$ 0

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

11. PUBLICATIONS DURING FY 1971

Oberjat, T., Zucker, R. and Cassen, B.: Rapid and Reliable Differential Counts on Dilute Leukocyte Suspensions. J. of Lab. and Clin. Medicine 76, 518-22, 1970.

Over 1200 reprint requests were obtained for the paper, Zucker and Cassen, The Separation of Normal Human Leukocytes by Density and Classification by Size, Blood 34, 591-600, 1969.

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 viable leukocytes; irradiated leukocytes, leukocytes in treated and untreated leukemia, leukocytes separated from the blood of irradiated rabbits and the effects of drugs on lymphocytes.

13. RELATIONSHIP TO OTHER PROJECTS

Particularly Dr. E. Cronkite, Brookhaven Natural Laboratory and Dr. Marvin Van Dilla, Los Alamos and many other investigators all over the world have become interested in these types of hematological investigations.

14. TECHNICAL PROGRESS IN FY 1971

Despite the reduction in personnel and budgetary support, significant progress has been made in FY 1971. Most interestingly, it has been found that a precision density gradient technique can be used to obtain viable basophil cell suspensions almost entirely free of neutrophils and eosinophils. Basophilic granulocytes typically comprise less than 1 per cent of the total leukocyte population in normal human blood.

In isotonic bovine serum albumin density gradients, basophils band in the same region as lymphocytes and are outnumbered by at least 20 to 1. Slightly increasing the osmolarity of the gradient medium will cause all cell types to lose water and increase in density. However, we have found that basophils are far more sensitive to hypertonicity than are lymphocytes. With increase of osmolarity basophils will band at a higher density than lymphocytes. It is now possible to accurately size the basophil fraction as a separated volume peak obtained by Coulter aperture sizing.

The basophil fractions are still contaminated with lymphocytes. These preparations are being studied for relative phagocytic activity and radiation sensitivity.

Less than 30% of the leukocytes layered in a bovine serum albumin density gradient at pH 7 are recovered as single cells after centrifugation. The losses are due to cell aggregation. We have found that this aggregation can be prevented even at pH 7 by the addition of G-acid (2-napthol - 6, 8 disulfonic acid, dipotassium salt). Dye exclusion tests indicate that cells remain viable after addition of the G-acid. In order to approach physiologic conditions as nearly as possible particular attention is being paid to the (Na+) to (K+) ratio.

15. EXPECTED RESULTS IN FY 1972 and FY 1973

This particular program will be phased out soon due to Dr. Cassen's retirement in 1972. In the interval prior to retirement - he will devote his major attention to work in the Instrumentation Program (Category 06-02-08) and the Biomedical Cyclotron Program (06-03-01).

The cell separation work is considered to be very important and we plan an orderly termination. Since the work relies on biophysical capabilities and procedures still being refined, the unfinished projects in this program which can be concluded is approximately FY 1972 will be carried out in budget category 06-02-08.

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: APPLIED MAMMALIAN RADIOBIOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location:
UCLA

7. Person in Charge:
John E. Byfield & Leslie R. Bennett, M.D.

8. Project Term:
From: 1970 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2	1 1/4	1 1/4
(b) Other Tech.	-	-	-
TOTAL:	2	1 1/4	1 1/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 16,800	$ 15,000	$ 16,000
(b) Materials and Services	3,000	2,500	5,000
Sub-Total Direct Project Support	$ 19,800	$ 17,500	$ 21,000
(c) Indirect Expenses *	8,300	8,200	8,500
TOTALS:	$ 28,100	$ 25,700	$ 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 1971

Byfield, J. E. and Y. C. Lee: The Effect of Synchronizing Temperature Shifts on the Synthesis and Translation of Replication-Supporting Messengers in Tetrahymena Pyriformis. Exptl. Cell Res. 61, 42-50, July 1970.

Byfield, J. E. and Y. C. Lee: Do Synchronizing Temperature Shifts Inhibit RNA Synthesis in Tetrahymena? J. Protozool. 17 (3), 445-453, August 1970.

Lee, Y. C. and J. E. Byfield: Characteristics of in vitro Ribonucleic Acid Synthesis by Macronuclei of Tetrahymena Pyriformis. Biochemistry 9 (20), 3947-3959, Sept. 1970.

Byfield, J. E., Y. C. Lee, and L. R. Bennett: Similarity of Mammalian and Tetrahymena RNA Polymerase Based on Rifampicin Resistance. Biochim. Biophys. Acta 204, 610, 1970.

Byfield, J.E., J. J. Stein, and L. R. Bennett: In Vitro Assay of Chemosensitivity in Gynecologic Malignancies: Technique and Preliminary Results Using a Radiochemical Microassay. Oncology 25 (1), 55-65, 1971.

Rangel, D. M., H. Neustein, J. E. Byfield, K. Shiroki, and E. W. Fonkalsrud: Ultrastructural Cytochemical Study of Lysosomes during Hepatic Ischemia in Dogs. Surg. Gynec. & Obstet. (in press).

Draz, S., J. E. Byfield, P. Byfield, and E. W. Fonkalsrud: Role of Liver Histiocytes in the Rejection of Tumor Cells. Arch. Surg. (in press).

Byfield, J. E. and Y. C. Lee: Effects of Cold-Shifts on the Translational Efficiency in Tetrahymena. Plant and Cell Physiol. (in press).

Byfield, J. E., Y. C. Lee, and L. R. Bennett: Sequential DNA Replication in Tetrahymena. J. Cell Biol. 47, 118a, 1970 (abstract).

Byfield, J. E., L. R. Bennett, and Y. C. Lee: Effects of Heavy Water on Transcription, Translation, and Cell Division in Tetrahymena Pyriformis GL. J. Cell Biol. 47, 28a-29a, 1970 (abstract).

Byfield, J. E., P. E. Byfield, J. D. Collins, and L. R. Bennett: Thermal Bone Marrow Expansion: Preliminary Results with Mice. Proc. Xth Inter. Cong. Radioprot. Grenoble, 1971 (abstract).

Byfield, J. E., P. E. Byfield, and L. R. Bennett: Preliminary Observations on in vitro Cancer Chemosensitivity Testing using Radioactive Tracers. Proc. 21st Annual Meeting, Tissue Culture Assoc., A-26, 1970 (abstract).

Thompson, R. W., J. E. Byfield, R. Kagon, and E. A. Langdon: Some Recent Advances in Radiation Therapy. Ann. Int. Med. (in press).

Bennett, L. R., F. Connon, and J. Felicissimo: Effect of Salt Stress on Tumor Incidence. Proc. XIXth Annual Meeting, Rad. Res. Soc., 1971 (in press).

Byfield, J. E.: Are the Genes Coding for Cytokinesis Late-Replicating and Located on the X-Chromosome in Mammalian Cells. (submitted).

Byfield, J. E.: Synergistic Neurotoxicity between Vincristine and Ionizing Radiation. (submitted).

Byfield, J. E., Y. C. Lee, and L. R. Bennett: A Direct in vitro Assay for Radiosensitization by Drugs. Radiol. (submitted).

12. SCOPE OF THE PROJECT

The work supported in this section includes several projects directed towards an understanding of the effects of ionizing radiation on mammalian animals and tissues. It also includes some basic studies on the effects of radiation on cell division in eukaryotes, together with continued studies on the possible long term effects of radiation damage on neoplasia and organism survival. Several aspects of this work are directed specifically towards increasing survival at the animal level. In addition, a portion of the time is devoted to improving the results of clinical radiation therapy through a rationalization of combined radiation therapy and anti-neoplastic drugs by the development of in vitro radiosensitization assays. In aggregate, the various projects are weighted towards improving the application of radiation at the organism level and to developing radioprotective measures within a technical range which may be realistically applicable at the human level.

13. RELATIONSHIP TO OTHER PROJECTS

Part of the work deals with the long term effects of radiation on organism survival and as such related closely to the studies currently under way within the division of Environmental Radiation. For example, the studies on repair of radiation damage to mitochondrial DNA, are directly relevant to environmental radiation in view of the ubiquitous nature of mitochondria in higher organisms and their importance in tissue repair. Similarly, the recent studies on the bonding of small molecules to DNA following ionizing radiation exposure relate directly to the experiments within the Radiation Chemistry division, although our own work is more directly concerned with the possible mutagenic effects of these new molecular lesions. Also relevant to other studies on environmental radiation are our experiments designed to determine the mechanism by which an increased osmotic load can decrease the incidence of tumor formation in both control and irradiated rodents.

However, a significant portion of the work now being done is more or less unique. Our experiments on thermal bone marrow expansion, which have demonstrated a new means by which radioprotection can be obtained through a relatively simple environmental manipulation, relate to the wide variety of investigations now being conducted, including studies on marrow stem cell kinetics, chemical radioprotection etc. They are, however, not being duplicated anywhere else in the world. Similarly, while a great deal of effort is being put into clinical studies on combined radiation therapy and chemotherapy of malignancy, there has not as yet been any effort made to place this method of cancer therapy on a quantitative basis through the development of in vitro assay systems such as is described in one of the references above.

Finally, we are continuing to devote a portion of the worktime to continued studies on the mechanism of life cycle control in the eukaryotic organism Tetrahymena. The references cited above related to this work describe the

current status of this investigation, and explain in detail the basis for our current interest in a late-replicating DNA fraction in the control of cytokinesis.

14. TECHNICAL PROGRESS IN FY 1971

(a) Thermal Bone Marrow Expansion: We have conducted a series of pilot experiments directed towards evaluating the possible clinical usefulness of expansion of the body's core marrow into distal limbs secondary to an increased ambient temperature. These studies indicate that chronic elevation of the ambient temperature causes an extension of active marrow into the long bones of the limbs of rodents. This has been shown by comparing Iron and Technetium scans in warmed animals versus controls and by quantitative analyses of bone-marrow-forming colony assays (Till and McCullock) from warmed and non-warmed mice. In addition, the LD50 Cobalt of warmed mice is increased as compared to controls. These results, not yet published, indicate that this manipulation may be capable of causing net increases in the total body marrow stem cell reserve.

(b) Radiosensitization: We have now completed a series of preliminary experiments directed towards developing a simple *in vitro* test for determining the radiosensitization properties of different oncolytic agents. Our goal is to develop an assay for clinical testing of individual patients' tumors *in vitro*. The procedure we use is based on the observations of Cleaver and Painter that hydroxyurea inhibits normal replication but has little effect on repair replication. To determine the radiosensitizing capability of each oncolytic drug, we have studied the effects of various concentrations of each agent on thymidine incorporation in hydroxyuric treated, irradiated mouse leukemia cells in tissue culture. Our experiments, not yet published, have confirmed that the degree of inhibition of net thymidine incorporation under these conditions measures inhibition of repair replication. This was shown by comparing the net incorporation data to specific inhibition of repair using both repair replication in alkaline CsCl gradients and strand rejoining in alkaline sucrose gradients. The data indicate that this approach is feasible for biopsied cells. Our preliminary data (reported thus far only in abstract) also show clearly that a screening assay for radiosensitizers different from the general screening tests used by the NCI will probably be required.

(c) Role of Liver Macrophages in Cancer Immunity: We have performed some preliminary experiments in collaboration with the UCLA Department of Surgery directed towards analysing the role of hepatic macrophages in cancer immunity. These results have been reported; thus far, it appears that liver macrophages can actively phagocytize cancer antigens but their ability for cytotoxicity is quite limited. This may partially explain why the liver is a more common source of cancer metasteses than is the lymphocyte-rich spleen. However, perfusion of the liver with normal lymphocyte preparations may be capable of altering the response. These experiments are in progress.

(d) Radiosensitization by Normal Metabolites: Dose-Response curves for colony formation in irradiated HeLa cells exposed to glyceraldehyde indicate that the latter compound can increase cell killing by irradiation. Since virtually all malignant cell lines have faulty carbohydrate metabolism, which allows partially degraded sugar fragments to accumulate, the data suggest that an evaluation of such compounds as clinical radiosensitizers is warranted.

(e) Salt-Stress Tumor Formation: Earlier studies on rats subjected to chronic salt stress demonstrated a significant reduction in spontaneous tumors compared to control animals. The mechanism of this protection is under study using white Swiss and AKR mice. It is hoped that these studies will determine

whether viral-induced malignant transformation can be modified or blocked by salt stress.

(f) Control of Cytokinesis: During the past year we continued our studies of cell division in heat-synchronized Tetrahymena. A detailed study of in vitro RNA nuclear synthesis was published. These experiments showed that Tetrahymena contain at least two distinct RNA polymerases which appear to be subject to different control mechanisms. Studies on the sequence of DNA synthesis during the cell cycle showed that starvation-synchronized cells show only partial synchrony in replication. In addition, studies concerned with the time of replication of specific DNA sequences during the cell cycle indicate that DNA synthesis is sequential in Tetrahymena and the sequence repeats in each life cycle. These results will be published in detail in the near future. We also studied the effects of heavy water on ribosome function in vitro and in vivo. D_2O is known to be partially thermal-protective in Tetrahymena. Our data indicate that this protection stems from an inhibition of thermal disruption of H-bond of the cytoplasmic ribosomes. This stabilization preserves the translational efficiency and allows cell division to occur at higher temperatures than in aqueous medium. These studies will also be published in detail later this year.

15. EXPECTED RESULTS IN FY 1972

The various projects outlined above will be continued. Our goal is to make each of these investigations available for clinical exploitation as soon as possible. In addition, we plan to add the following studies:

(a) Repair of Mitochondrial DNA: In collaboration with Dr. Y. C. Lee, we plan to determine the ability of mitochondrial enzymes to repair the damage caused by ionizing radiation. Damage to mitochondria appears as a regular feature in aging, and the data obtained may be directly relevant to the late effects of radiation exposure.

(b) Effects of Salt Concentration on Malignant Transformation In Vivo and In Vitro: To evaluate directly the possible role of salt stress in reducing tumor formation in vivo we plan to stress AKR mice, a strain subject to a well studied lymphoma diathesis. This system appears to be a reasonable model for evaluating the effects of various chronic salt exposure regimens in vivo. In addition, we plan to determine quantitatively the effects of increased tonicity on malignant transformation in vitro. To do this the hydrocarbon transformation assay developed by C. Heidelberber and colleagues will be used. These two complementary studies should allow a direct evaluation of salt stress on malignant transformation under controlled conditions.

(c) Life-cycle Analysis: Relative radiosensitivity of cell division-coding messengers. During the forthcoming year we plan to study the effects of thermal shifts on the synthesis of specific (enzyme) messengers. These studies are a logical extension of previous work which has been concerned with general studies on transcription and translation in Tetrahymena. We also plan to study the effects of radiation pulses during the synthesis of these same messengers in synchronized Tetrahymena. This should allow us to compare the effects of ionizing radiation on general cellular messengers for a contrast to the effects of identical pulses on the synthesis and translation of division-coding messengers. We will also initiate DNA/RNA hybridization studies during the pre-cytokinetic period in a preliminary attempt to identify the specific messengers coding for cell division. The reasoning behind this approach has been published (Byfield and Lee, J. Protozool. 17, 445 (1970); the latter

article also outlines in detail the usefulness of the Tetrahymena system in life-cycle analyses.

16. EXPECTED RESULTS IN FY 1973

We anticipate that by FY 1973 clinical trials of radiosensitization and thermal bone marrow expansion will be underway. The remaining studies should also be well enough advanced so that their potential usefulness in oncology can be evaluated.

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 - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	1/4	0	0
(b) Other Tech.	1/2	0	0
TOTAL:	3/4	0	0

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 7,700	$ 0	$ 0
(b) Materials and Services	2,200	4,000	4,200
Sub-Total Direct Project Support	$ 9,900	$ 4,000	$ 4,200
(c) Indirect Expenses *	8,300	0	0
TOTALS:	$ 18,200	$ 4,000	$ 4,200

* 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 1971

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 1971

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 1972 and FY 1973

Dr. Norman S. Simmons will be on sabbatical leave during FY 1972 and the scope of his GEN-12 research effort in FY 1973 and future years, has not been determined at this time.

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 - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	3	3	3
(b) Other Tech.	½	-	-
TOTAL:	3 ½	3	3

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 48,400	$ 41,000	$ 44,000
(b) Materials and Services	7,900	7,300	8,100
Sub-Total Direct Project Support	$ 56,300	$ 48,300	$ 52,100
(c) Indirect Expenses *	33,200	24,600	25,500
TOTALS:	$ 89,500	$ 72,900	$ 77,600

* 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 1971

M. E. Fewster, J. F. Mead, F. J. Wolfgram and W. W. Tourtellotte. Cholesterol Esters in Myelin Isolated from Cerebral White Matter of Patients with Multiple Sclerosis. Proc. Soc. Exp. Biol. Med. 133, 795 (1970).

S. V. Pande and J. F. Mead. Inhibition of the Stearyl CoA Desaturase System by Sterculate. J. Biol. Chem. 245, 1856 (1970).

G. A. Dhopeshwarkar and J. F. Mead. Fatty Acid Uptake by the Brain. III. Incorporation of 1-^{14}C Oleic Acid into the Adult Rat Brain. Biochim. Biophys. Acta 210, 250 (1970).

J. S. Robinson and J. F. Mead. A Potentially Unique Type of Lipid Storage in the Pink Salmon. Canad. J. Biochem. 48, 837 (1970).

J. F. Mead and D. F. Haggerty. Recent Advances in the Metabolism and Function of the Essential Fatty Acids. Excerpta Medica Nutrition. 8. (1970).

O. A. Schjeide, F. Galey, E. A. Grellert, R. I-San Lin, J. DeVellis and J. F. Mead. Macromolecules in Oocyte Metabolism. Biology of Reproduction, Vol. 2, No. 3, pp. 14-43, June 1970.

G. A. Dhopeshwarkar, C. Subramanian and J. F. Mead. Fatty Acid Uptake by Brain. IV. Incorporation of [1-^{14}C] Linoleic Acid into the Adult Rat Brain. Biochim. Biophys. Acta 231, 8 (1971).

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. S. Bickerstaffe, Dr. A. N. Davison), Japan (Dr. M. Kayama, Dr. M. Uchiyama).

14. TECHNICAL PROGRESS IN FY 1971

In a study of the brain alpha-oxidation system for long-chain fatty acids, it has been found that the mitochondrial system that degrades phytanic acid (and is absent in Refsum's disease) and probably sphingosine is quite different from the microsomal system that degrades brain long-chain fatty acids. The latter now appears to require only microsomes, 100,000xg supernatant and ferrous ion. Investigation of these requirements reveals that the iron requirement may reflect a non-heme iron protein while the supernatant requirement may be an ascorbic acid-containing peptide, which would, if proven, be the first case in which this substance acted in bound form.

The passage of fatty acids across the blood-brain barrier has been shown to occur rapidly for all fatty acids tried. Rather than a transport barrier, a saturation or exchange phenomenon appears to be involved in the incorporation of these fatty acids into the brain lipids. It occurs very rapidly (15 secs for acetate) and without the intervention of the liver, indicating that the form in which transport occurs must be the free fatty acids. Preliminary studies of the effect of x-irradiation (500-1000r) to the head have had equivocal results. Although an increase in incorporation of acetate into certain lipids was shown, the experimental design did not permit interpretation.

15. EXPECTED RESULTS IN FY 1972

During this year, the elucidation of the mechanism of alpha-oxidation should progress hopefully to reveal the nature of the enzymes and cofactors involved. The hydroxylation reaction should be investigated and the function of this system in brain development and aging should become clearer.

Understanding of the phenomena involved in the passage of fatty acids from the blood into the brain lipids should be advanced. With a knowledge of the mechanisms involved, the irradiation experiments can be repeated and, hopefully, interpreted in the light of transport or transferase reactions.

The metabolism of essential fatty acids by cells in culture will involve the use of microsomal suspensions of liver cells grown under different conditions with the aim of clarifying the control mechanisms involved.

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 1973, 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.:	4. Date Prepared:
06-01-02	April - 1971
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 1971	FY 1972	FY 1973
(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 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 38,400	$ 41,000	$ 43,000
(b) Materials and Services	8,400	7,400	8,200
Sub-Total Direct Project Support	$ 46,800	$ 48,400	$ 51,200
(c) Indirect Expenses *	24,900	24,600	25,500
TOTALS:	$ 71,700	$ 73,000	$ 76,700

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

11. PUBLICATIONS DURING FY 1971

NONE

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 1971

In discussing results of a detailed study of the γ-radiolysis of oleic acid (Howton and Wu, J. Am. Chem. Soc., 89, 523 (1967)), we suggested the possibility that during exposure of carboxylic acids in condensed states (liquid or solid, where such substances exist essentially entirely as carboxy-group-hydrogen-bonded "dimers") to ionizing radiation,

decarboxylation might proceed at least in part by a "cross-reaction" in which ionization of one member of such a pair of molecules actually leads to decarboxylation of the other. In an attempt to provide some experimental support for this proposed mechanism, we have looked carefully at the mole-ratio of penta- and heptadecanes formed by irradiation (dose ca. 93 Mrads) of equimolar mixtures of palmitic (P) and stearic (S) acids under two very different solid-state conditions. One sample was prepared by thorough admixture of the two finely-divided crystalline substances and thus consisted solely of self-paired "dimers": S-S and P-P. The other sample was prepared taking advantage of the well-documented fact (see Degerman and von Sydow, Acta Chem. Scand., 12, 1176 (1958)) that slow cooling of a melt of equimolar amounts of these two acids yields a "compound" in which all molecules are cross-paired, i.e., consist of S-P "dimers". The results were as follows:

nature of mixture	moles $C_{15}H_{32}$/moles $C_{17}H_{36}$ (± standard deviation)
self-paired	1.05 ± 0.04
cross-paired	0.96 ± 0.02

Since the experiment in which the two acids are self-paired demonstrates that the more extensive decarboxylation of the lower homolog more than offsets the consequences of this component representing a smaller electron-fraction of the "mixture", it follows from the predominance of the higher homologous hydrocarbon in the irradiated "cross-paired" sample that some sort of cross-reaction such as that proposed must be importantly involved.

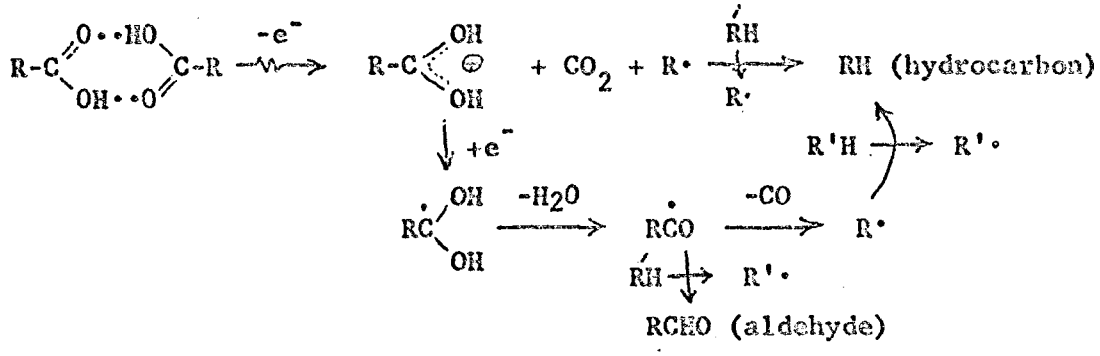
Consideration of the mechanism by which fatty acids irradiated in the solid state are decarboxylated suggests that the resulting crystalline material should contain trapped α-radicals (confirmed qualitatively by a variety of studies involving electron-spin-resonance spectrometry) which, when the crystal is dissolved in an inert solvent (or is melted), would be expected to couple, yielding α,α-linked "dimers", in quantity presumably equivalent to that of hydrocarbon (e.g., n-heptadecane from stearic acid) obtained. We have been able to show that such dimers are, in fact, produced, but have been unable to "trap" the precursor α-radicals by dissolving irradiated crystalline stearic acid in benzene containing iodine, or in cyclohexene or 1-hexene. It was concluded tentatively that the resonance-stabilized α-radicals might be unreactive toward the reagents employed. It has now been determined, however, that the yield of α,α-dimers is surprisingly low ($G = 0.164$ at 93 Mrads), i.e., about 1/10 that anticipated on the basis of amounts of hydrocarbon found concomitantly. Experiments are being designed currently to test the possibility that α-radicals are actually produced in quantity equivalent to decarboxylation but are extensively reconverted to stearic acid by reaction with thermal hydrogen atoms, which are presumed to have considerable mobility within the crystal lattice.

The work of Bennett and Gale (Trans. Faraday Soc. 64, 1174 (1968)) demonstrates that electrons react quite readily with carboxylic acids and thus that the corresponding aldehydes should be found among the radiolysis products of such substances. Earlier workers (see Newton, J. Chem. Phys. 26, 1764 (1957)) have noted the presence of such aldehydic products but, apparently in the belief that these were of minor

importance, they had never (to our knowledge) been quantitated. By modification of routine techniques which had caused us to be unaware of the presence of such products, we have now shown that, for example, stearaldehyde is indeed formed during radiolysis of stearic acid and, moreover, that it is present in quantity sufficient to justify its being classed as a major product. Comparison of yields of heptadecane and of stearaldehyde obtained by exposure of stearic acid to various doses of γ-radiation bear this out:

dose, Mrads	G_h	$G_{ald.}$
ca. 100	2.80	0.43
11.5	3.08	0.74
1.15	3.48	0.44

Since aldehyde would be expected to be more sensitive than hydrocarbon (the decarboxylation product) to radiolytic alteration, the net yield of this product falls relatively rapidly with increasing dose, and the comparative importance of reduction to aldehyde is thus obscured at high dosage. More interestingly, the relationship of $G_{ald.}$ to dose is seen to be biphasic; the decrease in $G_{ald.}$ at lower doses we interpret as indicating that aldehyde arises via more than one pathway (several are readily imaginable), at least one of which is secondary, i.e., involving two sequential radiolytic processes, of which that outlined below is of particular interest insofar as it involves an intermediate proposed in our "cross-reaction" decarboxylation mechanism:



It will be noted that some hydrocarbon is produced by such processes (in addition to that of decarboxylation per se) and that formation of water and of carbon monoxide, long recognized as carboxylic acid radiolysis products, are intimately associated with aldehyde formation.

15. EXPECTED RESULTS IN FY 1972

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 1973

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 (see D. M. Small, J. Lipid Res. 8, 551 (1967)). 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 myelin.

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 - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	1 1/4	1 1/2	1 1/2
(b) Other Tech.	1/2	1/2	1/2
TOTAL:	1 3/4	2	2

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 15,900	$ 22,000	$ 23,000
(b) Materials and Services	6,000	4,800	5,500
Sub-Total Direct Project Support	$ 21,900	$ 26,800	$ 28,500
(c) Indirect Expenses *	8,300	16,400	17,000
TOTALS:	$ 30,200	$ 43,200	$ 45,500

* 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 1971

J. C. Nevenzel. Occurrence, Function, and Biosynthesis of Wax Esters in Marine Organisms. Lipids, 5, 308-319 (1970).

R. F. Lee, J. C. Nevenzel, and G.-A. Paffenhöfer. Wax Esters of a Pelagic Copepod as Affected by Diet. Science, 167, 1510-1511 (1970).

R. F. Lee, J. C. Nevenzel, and A. A. Benson. Wax Ester Structure and Metabolism in Marine Copepods. Fed. Proc., 29, 899 Abs. [Abstract No. 3716] (1970).

R. F. Lee, J. C. Nevenzel, G.-A. Paffenhöfer, A. A. Benson, S. Patton, and T. Kavanagh. A Unique Hexaene Hydrocarbon from a Diatom. Biochim. Biophys. Acta, 202, 386-388 (1970).

R. F. Lee, J. C. Nevenzel, G.-A. Paffenhöfer, and A. A. Benson. The Metabolism of Wax Esters and Other Lipids by the Marine Copepod, Calanus helgolandicus. J. Lipid Res., 11, 237-240 (1970).

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 of plant waxes are in progress in the University of Glasgow (G. Eglinton) and Department of Biochemistry, Washington State University, Pullman, Wash. (P. E. Kolattukudy). Wax ester metabolism in animals is under investigation at the Hormel Institute, Austin, Minn. (H. Schlenk), Marine Biology Research Division, Scripps Institution of Oceanography, La Jolla, Calif. (A. A. Benson and J. C. Nevenzel), Pioneer Research Laboratory of the National Marine Fisheries Service, Seattle, Wash. (D. C. Malins), and Department of Fisheries, Hiroshima University, Japan (M. Kayama).

14. TECHNICAL PROGRESS IN FY 1971

Two significant findings came out of our participation in the cruise of the R/V ALPHA HELIX to Guadalupe Island, Mexico, last year. We established that in two additional families of mesopelagic fishes wax esters may be important lipid constituents. In the first family, that of the bristlemouths or Gonostomatidae, in two out of four species caught wax esters were a major constituent of the total lipids; the other two contained only traces of wax esters. This family is said to be numerically the most abundant in the oceans. Mrs. N. K. Menon is now completing the analysis of their lipids. All three species of the second family examined, the hatchet fishes or Sternoptychidae, were shown to contain significant amounts of wax esters (perhaps 10-20% of the total lipids).

The second new perspective opened by the ALPHA HELIX experience was the work with Mrs. E. A. Baker of Scripps Institution of Oceanography on fish lipases. We could only demonstrate low wax ester lipase activity in mesopelagic fishes, even in those species known to contain high amounts of wax esters. However, the pyloric caecum of the large epipelagic species Trachurus symmetricus, the jack mackerel, was a rich source of lipase activity. In subsequent work at La Jolla with the economically important anchovy (Engraulis mordax), also an epipelagic species feeding on zooplankton, the crude enzyme was isolated by centrifuging dilute phosphate buffer homogenates of fresh or frozen tissues at 27,000 x g and 2° C. It could be partially purified by dialysis or ammonium sulfate precipitation. The pH optimum for hydrolysis of wax esters was about 7.0. The pyloric caecum was the richest source of lipase activity: crude preparations were twice as active as those from liver and three times as active as those from red muscle; white muscle had no lipase activity.

Although the fish lipases were also active in hydrolyzing triglycerides, the wax-ester-splitting activity was about twice that for triglyceride hydrolysis; by contrast, the commercial porcine pancreatic lipase which served as a reference enzyme in 1 hr released about 3.5 times as much fatty acid per mg protein from triglyceride as from wax ester. Only triglyceride lipase activity has so far been detected in copepods. This

work will be presented at a symposium on wax ester metabolism in May, 1971, during the national meeting of the American Oil Chemists' Society at Houston.

15. EXPECTED RESULTS IN FY 1972

The survey of midwater fishes and invertebrates for wax esters will be extended to additional families and species. We also expect to begin a study of the lipids of fresh water copepods for comparison with marine species. A cooperative program with the Institute of Environmental and Evolutionary Biology at UCLA will enable us to begin research on temperature effects on lipid metabolism. We will look for changes in the activities of specific enzymes which control lipid metabolism. In larger invertebrate animals and fishes we will also look for changes in the polyunsaturated fatty acids of the phospholipids in the sarcoplasmic reticulum, which surrounds the muscle fibrils. The presence of unsaturated fatty acids is known to control both the Ca^{++} uptake by these membranes and its Ca^{++}-stimulated "extra" ATPase.

If the budget permits, wax ester metabolism will be studied with laboratory cultures of suitable algae or protozoans with the objective of elucidating the interconversions of fatty acids and long-chain alcohols and studying the regulation of alcohol and wax ester biosynthesis.

The wax esters of some of the marine organisms we have studied contain high proportions of polyunsaturated long-chain alcohols. We want to determine the structures of the more abundant of these by isolating the individual compounds, degrading them oxidatively, and identifying the fragments by gas chromatography in order to deduce the positions of the double bonds in the original alcohols. We expect to find close structural relationships with the fatty acids present.

16. EXPECTED RESULTS IN FY 1973

The investigation of temperature effects on lipid metabolism in the fresh water ecosystem will continue in conjunction with similar studies on the enzymes controlling the metabolism of wax esters and other lipid types in unicellular experimental organism in the laboratory. The survey of midwater marine animals for the presence of wax esters will also continue as new material becomes available.

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 - 1971

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 1971	FY 1972	FY 1973
(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 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 29,200	$ 31,000	$ 33,000
(b) Materials and Services	6,000	5,200	5,500
Sub-Total Direct Project Support	$ 35,200	$ 36,200	$ 38,500
(c) Indirect Expenses *	16,600	24,600	25,500
TOTALS:	$ 51,800	$ 60,800	$ 64,000

* 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 1971

A. J. Fulco. Temperature-Mediated Hyper-Induction of Fatty Acid Desaturating Enzyme. Biochim. Biophys. Acta 218, 558-560 (1970).

J. F. Quint and A. J. Fulco. Substrate Specificities for Fatty Acid Desaturation in Bacilli. Fed. Proc. (in press, June 1971).

12. SCOPE OF PROJECT

An intensive investigation of oxygen-dependent unsaturated fatty acid biosynthesis is being undertaken using several species of Bacilli as experimental organisms. It is hoped that this study will elucidate the following:

1.) The mechanisms by which temperature, oxygen and other environmental factors control or affect the rate of unsaturated fatty acid biosynthesis.

2.) The mechanism of hydrogen removal in desaturation and the factors which control the positional specificity of double-bond insertion.

3.) The roles played by unsaturated fatty acids, particularly as components of complex lipids in the metabolism and function of the bacterial cell.

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 (UCLA) 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 1971

Cultures of B. megaterium 14581 were used to study temperature effects since the desaturating enzyme of this species is not present in cultures growing at 33° but is readily induced by transferring these cultures to 20°. It has now been found that temperature mediates 4 apparently distinct control mechanisms affecting unsaturated fatty acid biosynthesis in B. megaterium. One point of control is the temperature-mediated hyper-induction of desaturating enzyme, that is, the induction of desaturating enzyme levels in cultures transferred from 30-35° to 20° which are well above levels of the same enzyme found in comparable cultures grown only at 20° (see Fulco, A.J., Biochim. Biophys. Acta 218, 558 (1970). A second temperature-mediated control is the in vivo irreversible inactivation of desaturating enzyme, a process which follows 1st order kinetics. The enzyme half-life at 33° is 17 min., at 20° about 30 min. and at 8°, several hours.

In addition to irreversible inactivation there is a very rapid but reversible inactivation of desaturating enzyme that is temperature-dependent. Thus, when a 20° culture is transferred to 33°, reversible

inactivation (reversible by transfer back to 20°) lowers desaturation rate by 1/2 every 3 minutes.

A 4th temperature-mediated control appears to affect the stability of the desaturase synthesizing system. When 20° cultures are transferred to 33°, desaturating enzyme continues to be synthesized but at a constantly decreasing rate. After corrections for the rate of enzyme inactivation (chloramphenicol controls) it was found that the decay of the capacity to produce desaturating enzyme at 33° followed strict 0-order kinetics. The data seem to rule out temperature-dependent decay of messenger to explain the loss of desaturase synthesizing capacity at 33° and amino acid incorporation studies rule against any overall decrease in the rate of protein synthesis. At present, the most likely explanation appears to be a temperature-mediated effect at the DNA level, perhaps by the synthesis or binding of a repressor.

A non-temperature related control of desaturation is mediated by oxygen. Not only is O_2 an obligate cofactor in the desaturation reaction, as previously reported, but it is also necessary for the induction of the desaturase. Thus, when a 33° culture is transferred to 20° and incubated under N_2, there is no synthesis of desaturating enzyme until O_2 is admitted.

Progress relating to the other goals mentioned can be summarized as follows:

1.) Exogenous palmitate-1-C^{14} is rapidly taken up by Bacilli (1-2 min) and shunted to 2 distinct pools. One pool is rapidly β-oxidized and converted to β-hydroxybutyrate in a process essentially complete in 30 min. The second pool, which is not subject to β-oxidation is utilized for desaturation and for incorporation into lipids. The flow of activity is from palmitate to activated palmitate to neutral lipids to phospholipids. Desaturation takes place at the level of activated palmitate.

2.) Chain-length specificity for desaturation is narrow, with palmitate the preferred substrate and with C_{17} and C_{18} chain-lengths much less active. There is no desaturation of chain lengths below C_{16}. B. negaterium can desaturate exogenously supplied 10-hexadecenoic acid to 5,10-hexadecenoic acid and hence differs from B. licheniformis only in lacking the latters' Δ^{10}-desaturase.

3.) Attempts to obtain cell-free desaturating systems from B. negaterium and B. licheniformis have met with little success, despite employing a variety of cell-breaking techniques (lysis, sonication, grinding). Only 1-2% desaturation could be obtained using 1-C^{14}-palmityl-CoA as substrate under various incubation conditions. Instead, most of the substrate was converted to β-hydroxybutyrate or recovered unchanged. We now fell that the real substrate for desaturation may be the acyl carrier-protein derivative rather than the CoA ester.

15. EXPECTED RESULTS IN FY 1972

We hope to:

1.) Reinvestigate cell-free preparations from B. negaterium and B. licheniformis for desaturation activity but using, rather than palmityl-1-C^{14}-CoA, palmityl-1-C^{14}-acyl carrier protein. This

will first require the isolation and purification of ACP followed by synthesis of the palmityl-1-C^{14}-ACP derivative.

2.) Investigate, in detail, the temperature-dependent "decay" of the desaturase synthesizing system in B. megaterium. The 1st step will be to determine whether a repressor is being synthesized at 33° and this can be done by incubating pre-induced cultures at 33° for various times with protein-synthesis inhibitors and determining whether such treatment has inhibited "decay" by delaying the synthesis of repressor.

3.) Determine the interrelated effects of chain-length, branching and preexisting double-bonds on the specificity of desaturation in various Bacilli desaturation systems (Δ^5, Δ^{10} and others). These will be in vivo experiments using C^{14}-tagged precursors.

16. EXPECTED RESULTS IN FY 1973

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: DEVELOPMENTAL REGULATION

3. AEC Budget Activity No.:	4. Date Prepared:
06-01-02	April - 1971
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 1971	FY 1972	FY 1973
(a) Scientific	4	4	4
(b) Other Tech.	½	1	1
TOTAL:	4 ½	5	5

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 52,600	$ 56,500	$ 57,500
(b) Materials and Services	21,400	18,900	21,600
Sub-Total Direct Project Support	$ 74,000	$ 75,400	$ 79,100
(c) Indirect Expenses *	33,200	41,000	42,500
TOTALS:	$ 107,200	$ 116,400	$ 121,600

* 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 1971

Maria W. Seraydarian, E. Sato, and I. Harary. Effect of Inhibitors on the Calcium Exchange of Heart Cells in Tissue Culture. J. Molecular and Cell Cardiology 1, 439-444 (1970).

Maria 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., 138, 414-423 (1970).

P. Padieu, I. Harary, and F. Barbier. Étude du Mécanism de Diffusion Solide-Liquide des Acides Aminés en Chromatographie par Échange d'Ions. Role des Hautes Pressions. Path.-Biol., 18, 1041-1057 (1970).

Hazel 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

Our attention in the broad area of differentiation and control of myosin synthesis has further narrowed to the factors that normally appear in postnatal serum which stimulate myosin synthesis and also to the cellular events correlated with the achieving of specific function. Thus we are investigating the presence and development of pre-myosin myoblasts and their change into functioning heart cells and the role of growth and cell division in the differentiation process.

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 are focusing on the synthesis as myosin.

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:

R. De Haan – The Development and Beating of Chick Heart Cells; Jaffe, Holtzer, Konigsberg – Development of Skeletal Muscle Cells In Vitro Systems; W. Hommaerts – The Role of Innervation in Myosin Development; W. N. Sperlakis – The Functioning of Heart Cells in Culture; S. Hauscka – The Role of Collagen in Skeletal Muscle Cell Development; H. Herrmann – The Development of Skeletal Muscle Cells in the Developing Embryo.

14. TECHNICAL PROGRESS IN FY 1971

The role of serum in the control of myosin levels in cultured heart cells was studied by attempts to purify the serum factors which support the myosin level and also by measuring the turnover of myosin under varying experimental conditions.

With the use of radioactive amino acids the rates of synthesis and degradation were followed in complete growth media (CGM), and media minus serum. Serum is necessary for synthesis. Within 2 days the rate of myosin synthesis dropped to 15% of the control. Serum is also necessary to protect myosin against degradation. The rate of degradation is vastly increased in serumless media. Cells incubated in CGM plus colchicine exhibit a degradation and synthetic rate similar to the controls. Protein synthesis inhibitors inhibit the synthesis of myosin and the increase of calcium activated ATPase. Actinomycin D begins to inhibit the synthesis of myosin 2 hours after its application to the cells. The 1/2 life of myosin in normal cells is 3.5 days.

We have discovered that the serum factor responsible for maintaining the calcium activated ATPase (which we showed to be equivalent to the concentration of myosin) is heat stable and probably nonprotein. Addition of boiled serum supernatant is sufficient to maintain the myosin level.

This serum factor is not concerned with the differential plating of heart cells but is directly involved in the incubation phase and maintains myosin heart cells no matter how the cells were attached to the petri dish. Further experiments with insulin and thyroxin were disappointing and we are forced to conclude that none of the hormones we have tried are as effective as the heat stable factor present in serum.

Evidence is accumulating that despite the increase in myosin, which is directly proportional to the increase in cell number and this dependent on cell division, that developed heart cells do not divide. Cultures treated to kill specifically only dividing cells do not exhibit the increase in myosin but on the other hand they also do not show the loss seen in serumless media. This indicates that the increase in myosin found in CGM which is dependent on cell division probably arises from the division of immature cells which must divide in order to develop into myosin producing heart cells.

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 1972

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

1. Isolate and identify the heat stable factor in serum. Follow the effect of this factor on the synthesis and degradation of myosin.

2. Attempt to isolate the primitive heart cells or myoblasts by differential centrifugation.

3. Follow the development of myosin synthesis in skeletal muscle cells.

4. Attempt to study the turnover of myosin messenger RNA.

16. EXPECTED RESULTS IN FY 1973

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: DEVELOPMENTAL NEUROBIOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

7. Person in Charge: Harvey Herschman

8. Project Term: From: 1970 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2	2	2
(b) Other Tech.	½	½	½
TOTAL:	2 ½	2 ½	2 ½

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 29,000	$ 30,500	$ 31,500
(b) Materials and Services	13,700	11,900	13,700
Sub-Total Direct Project Support	$ 42,700	$ 42,400	$ 45,200
(c) Indirect Expenses *	16,600	24,600	25,500
TOTALS:	$ 59,300	$ 67,000	$ 70,700

* 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 1971

J. Lightbody, S. E. Pfeiffer, P. L. Kornblith and H. Herschman. Biochemically Differentiated Clonal Human Glial Cells in Tissue Culture. J. Neurobiol. 1, 411 (1970).

H. Herschman, L. Levine and J. de Vellis. Appearance of a Brain Specific Antigen (S-100 Protein) in the Developing Rat Brain. J. Neurochem. (in press).

H. Herschman. S-100 Protein Accumulation in Developing Animals and Cell Cultures, UCLA Forum in Medical Sciences (in press).

C. Cotman, H. Herschman and D. Taylor. Subcellular Fractionation of Cultured Glial Cells. J. Neurobiol. (in press).

M. P. Lerner, F. O. Wettstein, H. R. Herschman, J. G. Stevens and B. R. Fridlander. Distribution of Polysomes in Mouse Brain Tissue. J. Neurochem. (in press).

S. E. Pfeiffer, H. Herschman, J. Lightbody, L. Levine and G. Sato. Cell Surface Antigenic Changes as a Function of Culture Conditions. J. Cell Physiol. (in press).

D. Hafeman, A. Costin, H. Herschman and C. Cotman. Effects of Enzymes, Complement, and Antisera on Evoked Potentials and Impedence. Int. Cong. of Physiology (in press). (Abstract)

12. SCOPE OF THE PROJECT

This project is concerned with the expression of macromolecular specificity in the nervous system. We have attempted to define neuronal and glial markers, both soluble and membrane associated, that are specific to neural tissue. Our objectives have been twofold; 1) to define the development of nervous-system specific functions in clonal cell populations under rigidly controlled conditions of cell culture, and 2) to characterize the appearance, nature, and hopefully function of nervous system specific macromolecules in the developing rodent brain. Previously we have characterized a nervous-system specific soluble protein unique to glial cells, (the S-100 protein), and we have isolated clonal cell populations which synthesize this protein in vitro. Now that the basic parametric studies on this protein have been completed (see previous FY publications) we have embarked upon a program, using radioisotopic tracer methods, designed to study the control of the synthesis and degradation of this protein in both clonal cell populations and developing neural tissue. By comparing the controlling factors on the synthesis and degradation of this organ-specific protein in clonal cultures of cells and in whole brain we hope to be able to evaluate the relative roles of intracellular regulatory phenomena and intercellular communication on the expression of organ specific neural gene products.

A parallel project in our laboratory has been concerned with the isolation and immunochemical characterization of central nerve ending particles. The objective here has been to immunologically define

membrane-associated neuron specific macromolecules, and then to isolate and characterize the components which give the nerve ending its unique characteristics. We also plan to utilize the antisera derived from such a study to probe the physiological role of the homologous antigenic structures in synaptic function, as well as their appearance in neural development, in conjunction with physiological and anatomical analysis. As an independent approach to macromolecular neural specificity we have recently begun an analysis of the glycoprotein synthesis of clonal glial and neuroblastoma cells in cell culture. This study will emphasize plasma membrane glycoproteins, and should complement the immunochemical approach described previously.

13. RELATIONSHIP TO OTHER PROJECTS

Our cell culture studies are closely related to those of Dr. Jean de Vellis, also of the Developmental Cell Biology Division. Dr. de Vellis is concerned with the regulation of other brain-specific macromolecules in these same clonal cell lines.

The studies on synaptosomal antigenic characteristics are being carried out in collaboration with Dr. Carl Cotman of the Department of Psychobiology, U. of California, Irvine. A number of laboratories are concerned with the purification and characterization of nerve ending membrane proteins. The physiological studies with this antisera carried out to date have been performed with members of the Physiology Department at UCLA, from support provided to Dr. Ross Adey.

14. TECHNICAL PROGRESS IN FY 1971

1. S-100 Protein in Clonal Cell Cultures

We have previously isolated two clonal cell lines which synthesize this protein [Lightbody, Pfeiffer, Kornblith, and Herschman, J. Neurobiol. 1, 411 (1970); Pfeiffer, Herschman, Lightbody, and Sato, J. Cell Physiol. 75, 329 (1970)]. The former, a human line, accumulates the protein during all phases of growth. The latter cell line, derived from a chemically-induced rat astrocytoma, does not accumulate the protein while growing, but only after becoming confluent on the culture plate. To investigate this phenomenon further, it has been necessary to devise an analytical procedure which can measure directly the incorporation of radiolabelled precursors specifically into the S-100 protein. The requirements for such an analytical technique are twofold; 1) it must be demonstrated to be specific and 2) quantitative recovery must be demonstrated.

In the past year we have devised a labelling and isolation procedure for S-100 protein which combines 1) antibody precipitation (in antibody excess) of the labelled protein, 2) dissolution of the antibody-antigen complex in sodium dodecyl sulfate (SDS) solution, and 3) electrophoresis on SDS containing acrylamide gels. The gels are stained for proteins, destained, sliced, dissolved and counted in a scintillation counter. Specificity can be demonstrated by showing that control cell lines (e.g. HeLa) processed in a similar fashion do not have labelled S-100 protein. Quantitative recovery is demonstrated by showing, by a second antibody-antigen precipitation and gel electrophoresis of the supernatant remaining after the first such precipitation of labelled glial cells,

that all labelled S-100 protein is removed in the first precipitation.

Utilizing this technique we have measured by pulse labelling the relative rate of synthesis of total acid precipitable protein and S-100 protein during a growth cycle of the clonal human glial cells. While total protein synthesis declines with increasing density, S-100 protein synthesis appears to be constitutive. We have also measured the degradation rate of S-100 protein and total acid precipitable protein in both clonal lines of cells, and shown that, in both cell lines this protein is degraded with a half life longer than total protein. A manuscript describing these results is in preparation.

2. Cell Surface Antigens of Clonal Glial Cells

We have previously described some of the culture-dependent surface antigens of the rat glial cells (Pfeiffer, Herschman, Lightbody, Levine and Sato, J. Cell Physiol. in press). During the past year we have also prepared antisera to the clonal human cell line. We have recently been interested in the contributions of sialic acid to the antigenic determinants of the surface membranes of mammalian cells, and have used these two clonal cultures as models for this analysis. In short, specific removal of sialic acid from either cell by enzymic digestion increases the reaction between the cultured cells and the homologous antiserum. This unexpected result implies that either 1) the antibody molecules are repelled from the cell surface prior to enzymic treatment due to the negative charge of the surface sialic acid, or 2) the sialic acid present on cell surfaces is removed by serum sialidase prior to antibody formation, exposing cryptic antigens which are masked prior to enzyme in the in vitro immunoassay. Studies on growth properties in relation to surface sialic acid, restoration of enzymically-removed sialic acid, etc. are currently in progress. The generality of this phenomenon in the serological analysis of carbohydrate containing antigens cannot be overemphasized. A manuscript describing these results is in preparation.

3. Antisera to Nerve Endings

In conjunction with Dr. Carl Cotman we have attempted to define the optimal isopycnotic separation conditions for synaptosomal isolation. We have subjected clonal glial cells to the various separation procedures commonly used for nerve ending particle isolation. These results (Cotman, Herschman, and Taylor, J. Neurobiol. in press) have shown that there is much less glial contamination in the synaptosomal region of ficoll-sucrose density gradients than in the corresponding region of sucrose gradients. We have also, as a result of this technique, devised a method for the isolation of the plasma membrane of these cells.

During the past year we have prepared antisera to ficoll-sucrose isolated synaptosomes. We have employed complement-fixation to demonstrate the organ specificity of this antisera, which reacts with rat nerve ending particles but not with membrane preparations from liver, kidney, spleen or heart. Similarly the organelle specificity of the antisera has been demonstrated; no reaction is seen with nerve ending particle mitochondria, whole brain mitochondria, brain nuclei, brain soluble protein, or membranes of clonal rat glial cells. Heat stability and resistance to enzymic digestion of the antigens have been characterized. Cross reaction of a variety of vertebrate nerve ending particle preparations have been quantitated. A manuscript describing these results is in preparation. Preliminary physiological experiments have shown definite

electrophysiological corrolates characteristic of iontophoretic application of this antiserum (Hafemann, Costin, Herschman and Cotman, in press).

15. EXPECTED RESULTS IN FY 1972

During FY 1972 we hope to resolve the question of the lack of accumulation of S-100 protein during exponential growth of the clonal rat glial cells. The radio-immune electrophoretic technique described previously should enable us to easily decide among the various alternatives. These results will be of great significance, I believe, since they will provide the beginning of a molecular understanding of a density-induced, contact mediated expression of an organ specific function, a basic problem in the expression of differentiated capacity. We will also measure such characteristics as S-100 specific messenger half life, the effect of ionic environment on S-100 synthesis and degradation, etc., and in general examine the regulatory controls concerned with synthesis and degradation of this specific neural protein. We plan to devise a similar assay for microtubule protein (a predominantly neural protein) and perform similar sorts of experiments on differentiating clonal neuroblastoma cells in culture.

A major portion of our time and effort will be devoted to the solubilization, isolation, and characterization of the synaptosome membrane antigen described above. We will begin with a variety of approaches including detergent, salt, solvent, and enzymatic extractions, and proceed as the data indicates.

16. EXPECTED RESULTS IN FY 1973

It is difficult to project to this date, since our work at this point will be dependent on two unknown factors; 1) the results of the preceding year, and 2) the level of funding available for the proposed research. Assuming the optimal availability of both variables, we will probably be interested at this point in the 1) physical characterization of the nerve ending antigens, 2) their physiological roles, 3) the isolation and characterization of glial and neuronal glycoproteins and 4) the synthesis and degradation of microtubule protein in clonal neuroblastoma under a variety of culture conditions which define various degress of differentiation.

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 CELL BIOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

7. Person in Charge: Lazaro E. Gerschenson

8. Project Term: From: 1972 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	0	1	1
(b) Other Tech.	0	$\frac{1}{2}$	$\frac{1}{2}$
TOTAL:	0	1 $\frac{1}{2}$	1 $\frac{1}{2}$

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 0	$ 15,500	$ 15,500
(b) Materials and Services	0	10,400	12,100
Sub-Total Direct Project Support	$ 0	$ 25,900	$ 27,600
(c) Indirect Expenses *	0	8,200	8,500
TOTALS:	$ 0	$ 34,100	$ 36,100

* 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 1971

Delia Casanello and L. E. Gerschenson. Some Morphological and Biochemical Characteristics of Isolated Rat Liver Cells Dissociated with Sodium Tetraphenylborate and Cultured in Suspension. Exp. Cell Res. 59, 283-290 (1970).

L. E. Gerschenson, Myrna Andersson, Jill Molson and T. Okigaki. Tyrosine α-Ketoglutarate Transaminase Induction by Dexamethasone in a New Adult Rat Liver Cell Line (RLC). Science 170, 859-861 (1970).

12. SCOPE OF THE PROJECT

The fundamental problem underlying this project relates to the regulation of the genetic expression in eukaryote cells by hormones.

A cell line (RLC), derived from adult rat liver, has been established in our laboratory and several cell sublines that grow in chemically defined medium containing very small concentrations of insulin ($\sim 10^{-9}$M) has been also established. They have been shown to be responsive to insulin and corticosteroids in a way similar to that of the liver of the whole animal.

Using this newly developed system, several intimately related problems will be studied:

1) The regulation of pyruvate kinase and tyrosine transaminase by insulin and corticosteroids.

2) The regulation of protein synthesis by both hormones.

3) The mechanism of action by which this regulation is elicited.

13. RELATIONSHIP TO OTHER PROJECTS

There is a strong relationship between this project and others throughout the world: in this laboratory, I. Harary, J. deVellis, and H. Herschman; at U.C.L.A., M. Davidson, and S. Kaplan; in the U.S.A., G. Tomkins (U.C.S.F.), B. Thompson (NIH), F. T. Kenney (Oak Ridge Natl. Lab.), A. Moscona (Univ. Chicago), and A. Rothstein (Rochester); and in France, B. Ephrussi, etc.

14. TECHNICAL PROGRESS IN FY 1971

Since this is a newly formed section, all 1970 accomplishments of this investigator were reported in the submission by Dr. I. Harary under the project title of Enzyme Chemistry.

15. EXPECTED RESULTS IN FY 1972

During this year it is expected:

1) To establish the role of insulin and dexamethasone in regulating the rates of synthesis and degradation of the mentioned enzyme.

2) To study the regulation of protein synthesis by the above mentioned hormones.

3) To establish a possible relation between the enzyme Glutathione Insulin Transhydrogenase, disulfide-sulfhydril exchanges and insulin mechanism of action.

16. EXPECTED RESULTS IN FY 1973

It is anticipated that emphasis will be shifted towards a more detailed examination of the mechanisms by which the hormones effect protein synthesis as well as the establishment of another liver cell lines and subsequent genetic studies through hybridization.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: DEVELOPMENTAL RADIOBIOLOGY

3. AEC Budget Activity No.:	4. Date Prepared:
06-01-02	April - 1971
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 1971	FY 1972	FY 1973
(a) Scientific	2 ½	2 ½	2 ½
(b) Other Tech.	½	½	½
TOTAL:	3	3	3

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 34,400	$ 35,500	$ 37,500
(b) Materials and Services	14,500	11,800	14,300
Sub-Total Direct Project Support	$ 48,900	$ 47,300	$ 51,800
(c) Indirect Expenses *	24,900	24,600	25,500
TOTALS:	$ 73,800	$ 71,900	$ 77,300

* 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 IN FY 1971

de Vellis, J. and D. Inglish: Effect of Ionizing Radiation on Hormonal Induction of Brain Enzymes in vivo and in Cultured Cells. IVth International Congress of Radiation Research, Evian, France, June 28, 1970, p. 227 (Abstract).

Schjeide, O. A. and J. de Vellis: Turnover of Brain Stem Molecular Constituents Following X-Irradiation of the Head. IVth International Congress of Radiation Research, Evian, France, June 28, 1970, p. 192 (Abstract).

de Vellis, J., D. Inglish, R. Cole and J. Molson: Effects of Hormones on the Differentiation of Cloned Lines of Neurons and Glial Cells in "Influence of Hormones on the Central Nervous System" D. Ford, Editor, Karger, Basel (in press, 1971).

Herschman, H. R., L. Levine and J. de Vellis: Appearance of a Brain-Specific Antigen (S-100 Protein) in the Developing Rat Brain. J. Neurochem. (in press, 1971).

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 mechanism 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. This is the induction of glycerolphosphate dehydrogenase by cortisol and the regulation of lactate dehydrogenase by norepinephrine. Cell lines are particularly suitable systems to study control mechanisms in the hormonal regulation of synthesis of these two enzymes. In a neuronal cell line we are studying the hormonal interactions which control the outgrowth of neurites and the expression of several parameters of neuronal differentiation. The effects of ionizing radiation on hormonally regulated glial and neuronal differentiation is also under investigation in vitro. Ultrastructural studies by means of the electron microscope are conducted in this laboratory while the electrophysiological studies of neurons and glial cells in culture are conducted in collaboration with investigations of the departments of Anatomy and Physiology at UCLA. Since the cell lines that we are using are established from tumor tissues, the radiation and drug studies may provide useful information for the design of treatments of human malignant tumors of the nervous system.

13. RELATIONSHIP TO OTHER PROJECTS

This work is generally related to investigators 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, and C. Tobias, University of California, Berkeley, R. Browson, University of California, Davis; (b) Biochemical and physiological studies of neurons and glial cells in culture: G. Sato and S. Varon, University of California, San Diego, M. Niremberg, N.I.H., G. Tocco, Naples, Italy, D. Schubert and M. Cohn, Salk Institute, La Jolla, California.

14. TECHNICAL PROGRESS IN FY 1971

Regulation of Enzymes in a Differentiated Rat Glial Cell Line. We reported previously that the brain specific induction of glycerolphosphate dehydrogenase (GPDH) by cortisol and lactate dehydrogenase (LDH) by norepinephrine has been obtained in a cloned line of astrocytes. Hormonal specificity for each induction has been studied. Corticosteroids closely related to cortisol induce GPDH; and only epinephrine and norepinephrine induce LDH. The structural requirement for induction has been determined using hormone analogs. GPDH induction is not mediated via cyclic AMP while LDH is. Theophylline, which prevents the enzymatic breakdown of 3',5' cyclic AMP potentiates the effect of exogenous cyclic AMP. The relationship of RNA and protein synthesis to the inductions was investigated. The evidence obtained suggests that hormonal control occurs both at the transcriptional and translational level. GPDH has been isolated from the brain and purified and its physico-chemical properties will be studied.

Influence of Hormones on the Outgrowth of Neuronal Processes in vitro. A cell line derived from the mouse neuroblastoma tumor C-1300 contains acetylcholinesterase, choline acetyltransferase and tyrosine hydroxylase, enzymes found in neurons. When the neuroblastoma cells are grown in monolayer culture some cells develop long neurite-like processes and resemble differentiated neurons. In the present study, the effect of thyroid hormones, growth hormone and cortisol on the differentiation of neuroblastoma cells was investigated. Numerous observations have shown that in vivo thyroid hormones, growth hormone and cortisol are required for the growth and maturation of the nervous system, including the formation of neurites. Cells were grown as monolayers in T_{30} Falcon plastic flasks containing 3 ml of nutrient medium. Thyroxine, growth hormone and cortisol were added in single, double or triple combinations for one week. After 5 to 7 days a 3 to 10-fold increase in the number of cells with neurites was observed in the cultures treated with all three hormones. In the presence of one or any two hormones there was a slight but not significant increase. The observed effect is blocked by high concentration of horse serum but not by fetal calf serum. The effect of the 3 hormones on cellular ultrastructure was investigated by electron microscopy. One of the main effects of the three hormones was to increase markedly the number of polysomes. The present data suggest that the action of these hormones on neuronal differentiation is coordinated. Cyclic AMP, a mediator in the action of several hormones, also affects the morphology of neuroblastoma cells.

Isolation and Fractionation of RNA from the Rat and the Rooster: A Comparison of RNA from Different Organs and Cultured Cells. RNA from the rat and rooster was analyzed by polyacrylamide gel electrophoresis. Of primary interest were comparisons of the RNA profiles of these two species and analysis of the effects of hormones on such profiles. Observations of apparent artifacts led to a more detailed study of the effects of aggregation, conformational isomerization, variations in extraction method and enzymatic degradation.

When analyzed on sucrose gradients, RNA extracted by aqueous naphthalene disulfonate and phenol at 0-8°C yields a 41S aggregate (or conformational isomer) of 28S ribosomal RNA. Denaturation with heating or dimethylsulfoxide prevents the appearance of 41S and causes changes in the relative amounts of smaller RNA molecules. Analysis by gel electrophoresis yielded no 41S RNA and showed no differences when heated.

Three different methods of extraction yielded different RNA profiles on acrylamide gels.

RNA from ribonuclease-treated liver homogenates showed differences from untreated RNA, suggesting that many of the minor RNA types may be specific degradation products.

RNA from the following sources were compared: rooster brain and liver, chick and hen liver, rat brain and liver, and cultured rat glial cells and mouse neuroblastoma cells. Definite qualitative differences were seen between rat and chicken RNA. No qualitative differences were seen between RNAs of different organs, ages or sexes of the same species, but cultured rat glial cells had two high molecular weight RNAs (5.7 and 13.0 x 10^6 daltons) not present in whole rat brain. The mouse neuroblastoma culture also contained the 5.7 x 10^6 dalton RNA. An RNA of 88,000 daltons was common to all preparations except nuclei, suggesting that this RNA is the previously observed smooth endoplasmic reticulum RNA. A number of RNA types were observed which have not been previously reported and which may not be degradation products, therefore deserving further study.

No new or unique RNA types were induced by hormonal treatments. However, differences were noted in incorporation of uridine into RNA fractions.

All results were used to evaluate the comparative approach to RNA analysis by gel electrophoresis. It is concluded that, in general, comparisons of RNA profiles are valid only for different RNAs extracted by rigorously identical methods, and that artifacts of aggregation, conformational isomerization and degradation must be carefully ruled out before assuming that a given size of RNA is of possible biological significance.

15. EXPECTED RESULTS IN FY 1972

Glycerolphosphate dehydrogenase (GPDH) has now been isolated and purified from brain. Purified GPDH will be used to immunize rabbits, from which hopefully a highly specific preciptating antibody will be prepared. Immunoradiochemical studies will be used to investigate the origin of induced GPDH in the astrocyte cell line and in the brain *in vivo*. These studies will select between these possibilities: (a) the increase in enzyme activity results from the conversion of an immunologically cross-reacting, catalytically inactive precursor to active enzyme, (b) the rate of synthesis of the enzyme is increased, (c) the rate of degradation of the enzyme is decreased. Lactate dehydrogenase (LDH) induction in the astrocyte cell line will be investigated similarly. But, first, however, lactate dehydrogenase will need to be purified. The mechanism by which cyclic AMP induces LDH and hence is the mediator of norepinephrine will be investigated. The induction potential will be measured during the various stages of the cell cycle of the astrocyte cell line. These cells have been synchronized, and G_1, G_2 and S phases have been determined. RNA metabolism will continue to be investigated in the normal and induced states (hormonal induced differentiation) of cultured

neurons and astrocytes. Studies on mRNA regulation will be carried out by first isolating polysomes and then dissociating mRNA from the ribosomes. Attempts will be made to pinpoint the exact site at which cortisol regulates protein synthesis at the translation level. This point was previously established by studies using metabolic inhibitors.

The hormonal induction of neurites by thyroid hormones, growth hormone and cortisol in cultured neurons will be studied in regard to the biochemical composition of the plasma membranes that are formed during this process. For instance, it will be determined if the key brain membrane enzymes, such as choline acetyltransferase, Na^+, K^+ activated ATPase, adenylcyclase, phosphodiesterase, increase in activity. By gel electrophoresis and immunochemical techniques it will be determined whether new proteins appear in the new neuronal membranes. Attempts will be made to determine the role of each of the three hormones in neurite outgrowth (all 3 hormones need to be present to obtain an effect). In astrocytes, the question will also be asked whether cortisol and norepinephrine induce new plasma membrane proteins along with the induction of GPDH and LDH.

The extreme radioresistance of the astrocytoma cell line will be investigated using RNA, proteins and specific enzyme inductions as markers. The processes of radiation damage, repair and recovery will be studied to try to find a clue to the mechanism of radioresistance of this cell type. The effect of ionizing radiation on the synthesis, distribution and metabolism of some neurotransmitters will be studied in cloned cultured neurons.

The metabolic significance of the hormonal induction of GPDH and LDH will be studied with respect to lipid, carbohydrate and protein metabolism. First, the effect of these hormones on uptake of amino acids, glucose and RNA precursors will be measured by using as radiolabelled substances, α-amino isobutyrate, deoxyglucose and uridine respectively. The first two are non-metabolites and are often used to measure uptake.

The previously reported observations of ultrastructural changes induced by hormones in cultures of neurons and astrocytes will be investigated by electron microscopic autoradiographic techniques.

16. EXPECTED RESULTS IN FY 1973

Attempts will be made to characterize more precisely the mechanisms of induction of GPDH and LDH. For this purpose an *in vitro* synthesizing system will hopefully be developed. Attention will also be given to events happening in the nucleus. Specific possible changes will be investigated by polyacrylamide gel electrophoresis of nuclear proteins. Assay of the various forms of RNA polymerases will be conducted on normal and induced cells, and also by the use of inhibitors specific to one of the types of RNA polymerase it could be determined which, if any, of the RNA polymerases play a role in the hormonal induction under study.

Studies on plasma membranes outlined and started in the previous year will continue with emphasis on the role of membranes in cell communication during differentiation and in glial-neuronal relationship. The effect of ionizing radiation on these processes will be investigated since the radiosensitivity of the developing brain still remains partially unexplained.

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.: 06-01-02

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

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

8. Project Term: From: 1947 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	4	3 1/4	3 3/4
(b) Other Tech.	1/2	1/4	3/4
TOTAL:	4 1/2	3 1/2	4 1/2

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 57,100	$ 50,400	$ 56,400
(b) Materials and Services	20,100	18,500	19,700
Sub-Total Direct Project Support	$ 77,200	$ 68,900	$ 76,100
(c) Indirect Expenses *	41,500	32,800	34,000
TOTALS:	$ 118,700	$ 101,700	$ 110,100

* 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 1971

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. 92 (9), 2868-2870 (1970).

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. II. Resolution of a Rapidly Decaying Transient Absorption in Cytosine Solutions. J. Am. Chem. Soc. 92 (9), 2871-2874 (1970).

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. 92 (9), 2875-2882 (1970).

Hüttermann, J., J. F. Ward, and L. S. Myers, Jr.: Electron Spin Resonance Studies of Free Radicals Formed from Orotic Acid. J. Phys. Chem. 74, 4022-4029 (1970).

Myers, L. S., Jr.: Free Radical Damage of Nucleic Acids and Their Components by Ionizing Radiation. Fed. Proc. (in Press).

Theard, L. M., F. C. Peterson, and L. S. Myers, Jr.: Nanosecond Pulse Radiolysis Studies of Aqueous Thymine Solutions. J. Phys. Chem. (Accepted).

Hüttermann, Jürgen, John F. Ward and L. S. Myers, Jr.: Electron Spin Resonance Studies of Free Radicals in Irradiated Single Crystals of 5-Methyl-cytosine. Int. J. Rad. Phys. and Chem. (Accepted).

Zimbrick, J. D. and L. S. Myers, Jr.: EPR Studies on Trapped Species Produced in the Gamma Radiolysis of Aqueous Sugar Ices. J. Chem. Phys. (Accepted).

Johnson, L. A., and L. S. Myers, Jr.: The Gamma Radiolysis of Frozen Aqueous Thymine Solutions. Radiation Research 43, 256-257 (1970) (Abstract).

Zimbrick, J. D., and L. S. Myers, Jr.: Electron Paramagnetic Relaxation of Organic Free Radicals Trapped in Irradiated Frozen Aqueous Glucose Solutions. Radiation Research 43, 256 (1970) (Abstract).

Myers, Lawrence S., Jr., Leslie M. Theard, Frank C. Peterson, Mary Lynn Hollis, Aida Warnick, and Michelle Meyers: Spectra and Reactions of Free Radicals Formed by Pulse Radiolysis of Nucleic Acids and Related Compounds. IVth International Congress of Radiation Research, Evian, France, p. 154 (1970) (Abstract).

Theard, Leslie M., Frank C. Peterson, Richard L. Voigt, and Lawrence S. Myers, Jr.: Pulse Radiolysis Studies of Aqueous Solutions of Aminothiols and Compounds Related to Nucleic Acid. IVth International Congress of Radiation Research, Evian, France, p. 218 (1970) (Abstract).

Zimbrick, John D., John F. Ward and Lawrence S. Myers, Jr.: Studies on the Chemical Basis of Cellular Radiosensitization by 5-Bromouracil Substitution in DNA. IVth International Congress of Radiation Research, Evian, France, p. 239 (1970) (Abstract).

Myers, L. S., Jr., M. Meyers, L. M. Theard, and F. C. Peterson: Sites of Attack by OH Free Radical on Nucleotides and DNA: A Pulse Radiolysis Study. Annual Meeting of the Biophysical Society (1971) (Abstract).

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 those of J. E. Byfield, Julian Van Lancker, L. R. Bennett, and 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, University of Newcastle-upon-Tyne, England; G. E. Adams, Mount Vernon Hospital, England, L. M. Theard, Gulf Radiation Technology, a division of Gulf Energy and Environmental Systems Incorporated, San Diego, California; E. Hayon, U. S. Army Natick Laboratories, Natick, Massachusetts; (EPR) J. D. Zimbrick, University of Kansas, Lawrence, Kansas; A. Müller and J. Hüttermann, University of Regensburg, 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; W. Garrison, University of California, Berkeley, California; H. Loman and J. Blok, Vrije Universiteit Amsterdam, The Netherlands, and Kendric C. Smith, Stanford University, Palo Alto, California.

14. TECHNICAL PROGRESS IN FY 1971

Free Radical Damage of Nucleic Acids and Their Components by Ionizing Radiation: An extensive review of this subject has been prepared and will appear in Federation Proceedings in the Spring of 1971. The abstract, which indicates the scope of the paper, follows: Nucleic acid free radicals are important intermediates in a chain of events leading from depositions of energy by ionizing radiation to appearance of the biological effects of radiation. They are formed by deposition of energy directly within the nucleic acid, and by reactions of nucleic acids with hydrogen atoms, hydroxyl free radicals, and hydrated electrons produced by deposition of energy in water. On irradiation of pure nucleic acids different radical species are formed under different conditions. Temperature, base composition, moisture content, protein contamination, and possibly secondary structure appear to be important parameters. The 5,6-dihydrothymin-5-yl radical is present in many irradiated DNA samples. Hydrogen atoms and hydroxyl free radicals in aqueous media add to the base and abstract hydrogen from the pentose moieties of nucleic acids to give free radicals localized on the moiety attacked. The yields of base radicals are approximately proportional to the relative amounts of each base in the nucleic acid. Radicals formed by nucleic acid constituents undergo rapid secondary reactions with oxygen and certain other small molecules. Proposed chemical mechanisms for the sensitization of nucleic acids to radiation by 5-bromouracil and N-ethylmaleimide, and for protection by sulfhydryl compounds are discussed.

Pulse Radiolysis of Nucleic Acid Constituents: (All pulse radiolysis work has been done in cooperation with Dr. L. M. Theard, Gulf Radiation Technology, San Diego, California.) Studies of reactions between nucleic acid constituents and the products of water radiolysis (hydrogen atoms (H·), hydroxyl free radicals (·OH), and hydrated electrons (e^-_{aq})) are being carried out to provide a basis for understanding the mechanisms by which radiation damages nucleic acids. Previously we have reported on spectra and reactions of pyrimidine base radicals present during the period 3 to 1000 μsec after absorption of a short pulse of radiation by an aqueous solution. We have now used the nanosecond time resolution capability of our pulse radiolysis system to study the rates of formation and some of the reactions of these radicals during the period 0.010 to 4 μsec after the pulse. The rate constants for reaction of ·OH with nucleic acid bases, nucleosides and nucleotides have been obtained by direct observation of the formation of the organic free radicals. Values are strikingly similar, ranging from 4.5 to 6.4×10^9 M^{-1} sec^{-1} for the entire series of compounds, and are slightly larger for deoxynucleotides than for ribonucleotides. These values are significantly higher than those reported by other workers on the basis of competition kinetic methods, they show much less scatter, and are believed to be considerably more reliable. Other reactions which have been investigated include: a) reaction of O_2 with the (thymine-OH)· adduct radical ($k = 1.9 \times 10^9$ M^{-1} sec^{-1}); b) reaction of H· with cytosine, cytidine, thymine, and thymidine ($k = 3.4$, 3.4, 6.9, and 6.8×10^8 M^{-1} sec^{-1} resp.); c) reaction of H_3O^+ with anion radicals of thymine, and thymidine, i.e. protonation of products formed by reaction of e^-_{aq} with thymine and thymidine ($k = 6.4$ and 5.2×10^{10} M^{-1} sec^{-1} resp.); d) reaction of T^- anion radical with H_2O ($k \leq 10^4$ M^{-1} sec^{-1}); e) reaction of O_2 with T^- ($k = 4.3 \times 10^9$ $M^{-1}sec^{-1}$), f) rates of decay of 1) ·OH adduct radicals, 2) anion radicals; and g) rates of reaction of anion radicals with ·OH adduct radicals. The latter two investigations made use of the μsec time resolving capability of the system.

Pulse Radiolysis of DNA: Continued work on this project has led to an increased understanding of the site of attack by ·OH on DNA. Optical absorption spectra of free radicals formed by reaction of ·OH with DNA and DNA constituents were determined by pulse radiolysis of N_2O saturated solutions. Spectra of nucleotide radicals are almost identical to spectra of the corresponding bases. The extinction coefficients of the purine nucleotide radicals at wavelengths near ϵ_{max} are larger than those of the pyrimidine radicals by a factor of as much as 5. Deoxyribose phosphate radicals have an extinction coefficient which is much smaller than that of radicals formed by any of the bases or nucleotides. The spectrum of radicals formed by reaction of ·OH with DNA is consistent with attack by ·OH on the bases in DNA in proportion to the amount of each base present, but with radicals centered on the purine bases accounting for most of the absorption. This correlation does not exclude the possibility of attack on the sugar moiety. Such attack gives radicals whose absorption is too weak to contribute significantly to the absorption spectrum.

Pulse Radiolysis of Aqueous Solutions Containing DNA Plus a Second Reactive Compound: Modification of Effects of ·OH on DNA: This project is part of an attempt to investigate secondary reactions between organic free radicals and organic molecules. Such reactions must occur when living organisms are irradiated (see Item 15 for a more detailed explanation). Preliminary experiments with the radiation protective sulfhydryl compounds (RSH) such as cysteine and cysteamine have shown that the reactivity of ·OH towards them is approximately twice as great as toward pyrimidine bases, $k_{OH + RSH} = 1 \times 10^{10}\ M^{-1}\ sec^{-1}$, $k_{OH + T} = 5 \times 10^{9}\ M^{-1}\ sec^{-1}$. The product depends on pH. In slightly acid solution RS· radicals are formed. These have an absorption spectrum with a weak maximum at 310 nm. In slightly alkaline solutions $RSSR^-$ anion radicals, which have a comparatively intense absorption ($\lambda_{max} = \sim 400$ nm), are formed.

ESR Studies of Free Radicals Formed from the Nucleic Acid Pyrimidine Base Analog, Orotic Acid: Free radicals formed by irradiation of single crystals and polycrystalline materials, and by reaction with chemically prepared ·OH in aqueous solutions have been studied. The principal radicals observed in the solid state are a) a radical with a hydrogen atom added at the 5 position of the pyrimidine ring leaving an unpaired electron localized mainly on carbon $C_{(6)}$, and b) a radical with a hydrogen atom added to an oxygen of the carboxyl group giving rise to interaction of the radical electron on carbon $C_{(7)}$ with two hydroxyl protons. The ·OH radical adds to the 5-position of orotic acid and the unpaired electron is localized on carbon $C_{(6)}$. (In collaboration with Drs. J. Hüttermann (Postdoctoral Scholar) and J. F. Ward, Sub-Cellular Radiobiology Section.)

ESR Studies of Free Radicals in Irradiated Single Crystals of 5-Methylcytosine: Irradiation at 77°K followed by measurement at 130°K shows the presence of an anion radical. Irradiation and measurement at room temperature show the presence of two radicals: a) one with a hydrogen atom added to carbon $C_{(6)}$ of the pyrimidine ring, and b) one with a hydrogen atom removed from the 5-methyl group. Observation of these radicals in 5-methylcytosine confirms the assignment of spectra observed with irradiated thymine to analogous radical structures. The accumulated evidence from this and earlier work strongly suggests that the radicals, including the ones with an added hydrogen atom, have ionic precursors. (In collaboration with Drs. J. Hüttermann (Postdoctoral Scholar) and J. F. Ward, Sub-Cellular Radiobiology Section.)

EPR Studies on Trapped Species Produced by Gamma Radiolysis of Aqueous Sugar Ices: The yields, EPR spectral characteristics, and paramagnetic relaxation of trapped electrons (e_t^-) and sugar radicals were studied in ^{60}Co γ-irradiated monosaccharide ices at 77°K. The e_t^- yields in the various ices ranged from 0.95 to 1.8 and were strongly dependent on solute molarity and phase of the ice matrix. In most cases the sugar radical yields were the same as the e_t^- yields. The e_t^- could be bleached with white light to form dielectrons as previously found for e_t^- in alkaline ices. The EPR lineshapes, linewidths and line broadening mechanisms of e_t^- in the sugar ices were similar to the corresponding characteristics of e_t^- in alkaline ice. Spectral studies on the trapped sugar radicals indicated that two groups of radicals were present. The variation of the e_t^- and sugar radical relaxation times with radiation dose was studied in H_2O and D_2O matrices. The results for e_t^- were interpreted as indicating a spatially nonuniform distribution of this species; the spatial nonuniformity is not as great in the D_2O matrix as in the H_2O matrix. The results for the sugar radicals suggested that one group was distributed uniformly whereas the other group was distributed nonuniformly to the same degree as the e_t^- distribution. (In collaboration with Dr. J. Zimbrick, USAEC Postdoctoral Fellow.)

Other Studies: The following are being investigated: a) ESR studies of irradiated nucleic acids and nucleic acid constituents with emphasis on the role of electrons and ion radicals, b) Gamma radiolysis of frozen aqueous solutions of nucleic acids and nucleic acid constituents using optical spectroscopy, product analyses, and ESR spectroscopy to investigate intermediate reactions of electrons and ·OH, (in part in collaboration with Dr. G. D. Venerable, USAEC Postdoctoral Fellow), c) Splitting of pyrimidine dimers by ionizing radiation, d) Irradiation of freeze dried bacteriophage T, (in collaboration with Drs. Hazel Lewis, Postdoctoral Scholar, and J. F. Ward), and e) Preparation for exploratory experiments with the UCLA cyclotron when it goes into operation.

15. EXPECTED RESULTS IN FY 1972

Investigations of the radiolysis of nucleic acids and their constituents will be continued. Pulse radiolysis, ESR spectroscopy, and other techniques will be used to study mechanisms of action of radiation, both direct and indirect on nucleic acid constituents, nucleic acids, and nucleoproteins. Specific projects follow:

Pulse Radiolysis of Aqueous Solutions Containing a Nucleic Acid or a Nucleic Acid Constituent Plus a Second Reactive Compound: Modification of Effects of ·OH on Nucleic Acids: With the background gained from studies of simple systems and preliminary studies with two component systems, it is now possible to use complex systems to study reactions of a) organic free radicals with nucleic acids, b) nucleic acid free radicals with organic molecules, and c) nucleic acid radicals with organic radicals. Such reactions almost certainly occur in irradiated living cells, and are very likely an important source of biological damage. The action of chemical modifiers of radiation effects, such as sulphydryl compounds, N-ethylmaleimide, and oxygen also may involve such reactions. Experiments will consist of exposing to a radiation pulse an N_2O saturated solution containing both a nucleic acid (or constituent) and a second compound, and observing changes in absorption spectra and rate of decay of radicals. Concentrations can be varied so that ·OH will react predominantly with one or the other of the components, or equally with the two. Depending on the concentration ratio reactions a, b, or c above can be observed.

Pulse Radiolysis Study of Interactions Between Purine and Pyrimidine Base Anion Radicals and 5-Bromouracil (BU): We have previously advanced a hypothesis for a mechanism by which BU incorporation in DNA sensitizes the DNA to radiation effects. The hypothesis involves reaction of an electron with BU. By means of pulse radiolysis we hope to test a possible step in the hypothesis: transfer of the electron from a base anion radical to BU.

ESR Studies of Frozen Aqueous Solutions of Nucleic Acids and Nucleic Acid Constituents: In frozen solutions irradiated at low temperature (77°K) electrons and ·OH are trapped. On warming somewhat these species are released and under favorable conditions react with solutes incorporated in the ice to give long-lived organic radicals. Reactions with nucleic acids and nucleic acid constituents will be investigated, with particular emphasis on determining the structures and fates of radicals formed by the electron.

ESR of Irradiated Nucleic Acids and Nucleic Acid Constituents: A survey of the literature shows that at least seven distinctive ESR spectra have been reported for irradiated nucleic acids, and in many cases spectra have not been associated with radical structures. Experiments will be done with hopes of determining the influence of purity, protein contamination, secondary structure, temperature, moisture content, etc. on radical formation. Particular attention will be given to trapping and reactions of electrons, and to formation of cationic and anionic radicals.

Irradiation of Freeze Dried Bacteriophage T_1: In collaboration with Drs. Hazel Lewis (Postdoctoral Scholar) and J. F. Ward (Sub-cellular Radiation Biology) this project will be continued.

Other Projects: Numerous exploratory projects will be carried out. These include a) continued attempts to determine the mechanism of splitting pyrimidine dimers by ionizing radiation, and b) experiments with 22 MeV protons, 11 MeV deuterons, and energetic He^{3+} and He^{4+} ions produced by the new UCLA cyclotron. Radical formation and other radiation effects of these particles will be investigated and results will be compared with effects of γ-rays on the nucleic acid systems we have been investigating.

16. EXPECTED RESULTS IN FY 1973

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 viruses and cells. Specific problems will depend on the results of the preceding year's work. The following probably will be of importance: a) pulse radiolysis studies of secondary and subsequent reactions of nucleic acid radicals resulting from ·OH attack, b) investigations of methods of modifying these reactions, c) research with increased emphasis on reactions of electrons in solution (pulse radiolysis) and solid state (ESR), d) if exploratory experiments with cyclotron produced particles are promising, an extensive investigation of the action of these particles at the molecular level, and e) studies of the role played by protein in radiation damage of nucleic acids.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: SUB-CELLULAR RADIOBIOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

7. Person in Charge: John F. Ward

8. Project Term: From: 1964 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2 ½	3	3
(b) Other Tech.	½	1	1
TOTAL:	3	4	4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 30,700	$ 44,300	$ 45,300
(b) Materials and Services	11,700	13,800	14,600
Sub-Total Direct Project Support	$ 42,400	$ 58,100	$ 59,900
(c) Indirect Expenses *	16,600	32,800	34,000
TOTALS:	$ 59,000	$ 90,900	$ 93,900

* 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 1971

Hüttermann, J., J. F. Ward and L. S. Myers, Jr.: Electron Spin Resonance Studies of Free Radicals Formed from Orotic Acid, J. Phys. Chem. 74, 4022-4029 (1970).

Ward, J. F. and I. Kuo: The Effect of Chloride Ions on the γ-Radiation Induced Destruction of DNA, Nucleosides and Nucleotides in Aqueous Solution, Int. J. Rad. Biol. 18, 381-391 (1970).

Ward, J. F. and I. Kuo: The Effect of Radiation Modifiers on Sugar Phosphate Bond Breakage in Deoxynucleotides Irradiated in Aqueous Solution. IVth International Congress on Radiation Research, Evian, France, Abstract 908 (1970).

Zimbrick, John D., John F. Ward and L. S. Myers, Jr.: Studies on the Chemical Basis of Cellular Radiosensitization by 5 Bromouracil Substitution in DNA. IVth International Congress on Radiation Research, Evian, France, Abstract 937 (1970).

Ward, J. F.: Mechanism of Radiation Produced Single Strand Break Production in DNA, Biophysical Society Abstract TPM-C4 (1971).

Hüttermann, J., J. F. Ward and L. S. Myers, Jr.: Electron Spin Resonance Studies of Free Radicals in Irradiated Single Crystals of 5 Methylcytosine. Int. J. Rad. Phys. and Chem. (Accepted).

Ward, J. F.: Deoxynucleotides - Models for Studying Mechanisms of Strand Breakage in DNA. 1. Protection by Sulphydryl Compounds. Int. J. Rad. Phys. and Chem. (Accepted).

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 1971 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.

In conjunction with Dr. H. L. Lewis and Dr. L. S. Myers, Jr. work is being carried out with T_1 bacteriophage which is used as a model nucleoprotein system in attempts to assay differences between "direct" and "indirect" effects of radiation.

With Dr. J. Rudé studies on the ultraviolet irradiation of Escherichia Coli B/r are aimed at investigating the mechanisms of "Liquid Holding Recovery" in order to form a basis for studying split dose effects.

We are beginning to extrapolate our findings from model systems to cellular systems and we hope by this means to learn if it is possible to control the extent of DNA damage in living cells.

13. RELATIONSHIP TO OTHER PROJECTS

This work is closely related to the investigations in this laboratory carried out under the direction of L. S. Myers, Jr. and D. R. Howton and generally so to Julian Van Lancker, J. E. Byfield, E. H. Strickland and others. 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, Institut 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 1971

Deoxynucleotides: Model Systems for Investigation of the Mechanism of Production of Strand Breaks in DNA by Irradiation: The measurement of DNA strand breaks is currently used as the method of estimating radiation damage to cellular systems. We are using deoxynucleotides as simple model systems to investigate the molecular mechanisms of strand breaks. In the model system, breakage of the sugar phosphate bond is the type of reaction which will cause a strand break in DNA. Several significant advances have been made in understanding strand breaks using these model systems.

1. Primary event leading to strand breakage: In aqueous solution the radiation produced species responsible for strand breaks is the hydroxyl radical ($\dot{O}H$). This has been determined by use of radical scavengers and by use of nitrous oxide to convert hydrated electrons to $\dot{O}Hs$. The $\dot{O}H$ reacts with the deoxyribose moiety abstracting a hydrogen atom and producing a sugar radical which reacts further to produce a strand break. The sugar radical produced by this mechanism is the same as that which would be formed by direct ionization of the sugar molecule followed by loss of a proton. Thus the primary events which lead to strand break production whether occurring by "indirect" or "direct" mechanisms produce similar strand break precursors. In experiment designed to estimate the effect of base damage on sugar-phosphate bond breaka

radiation products of deoxynucleotides which did not contain phosphate were isolated. All these products retained their characteristic ultraviolet absorption, showing that damage to bases does not lead to release of inorganic phosphate and therefore is probably unimportant in producing strand breaks.

2. Subsequent reactions of the sugar radical: Sugar radicals react with oxygen to produce peroxy radicals and ultimately carbonyl compounds with breakage of the sugar phosphate bond. In the absence of oxygen, sulfhydryl compounds react with the sugar radical, donating a hydrogen atom and repairing the radiation damage. This result was used to investigate the mechanism by which the radical decays in the absence of oxygen. It could decay by either a bimolecular dismutation reaction or by a unimolecular decomposition. In the absence of oxygen, sulfhydryl repair reactions compete with the other decay reactions of the sugar radicals. Variation of dose rate showed that the mechanism of decay of the sugar radical is a unimolecular reaction probably occurring via elimination of water. Thus, the identity of the products of the sugar radical decay are dependent on the presence or absence of oxygen. This can be seen by the ratio of the yield of labile phosphate to that of inorganic phosphate which is dependent on whether oxygen is present or not. However, the total yield of phosphate is independent of the presence of oxygen and is dependent solely on the number of hydroxyl radicals reacting with the sugar moiety.

3. Products formed as a result of "strand breakage": Experiments with thymidine 3'5' diphosphate have shown that radical damage to the deoxyribose moiety results in the loss of both phosphate groups - no monophosphate radiation products could be found. This reaction occurring within a polynucleotide chain would result in the release of a monomeric unit and the formation of both a 3' and a 5' phosphate end group on the polynucleotide chain. If the damage to the sugar molecule is in the form of a labile strand break this would leave a "dirty" end on the strand break. Again, using nucleotide model systems the chemical identity of the monomer unit which would be released is being investigated. Using column chromatography these products have been isolated from all deoxynucleotides. So far it has been shown that all products have the conjugated ultraviolet absorbing system of the base intact. Examination of the ultraviolet spectra as a function of pH shows that there is no ionizable hydrogen associated with the 1 nitrogen of the pyrimidine and the 7 nitrogen of the purines. Thus, a part of the sugar molecule is still attached to the base molecules. Further investigations designed to characterize these products chemically are in progress.

Irradiation of Freeze Dried Bacteriophage T_1: This work is being done with Dr. H. L. Lewis, a Postdoctoral Scholar, with the collaboration of Dr. L. S. Myers, Jr. The bacterial virus T_1 is being used as a model nucleoprotein system. T_1 phage can be irradiated dry and in the presence of small amounts of moisture as well as in solution. Exposure to atmospheric moisture before ionizing irradiation increases the radiosensitivity of freeze-dried T_1 bacteriophage by a factor of 2. The characteristics of the hydration effect are (1) Maximum sensitization occurs at low water vapor pressure (0.9 and 1.5 torr, depending upon the medium). (2) The presence of SH compounds during irradiation eliminates the effect. (3) The absolute sensitivities depend upon the medium from which the T_1 is freeze-dried. (4) At maximum sensitization the hydrated T_1 is more resistant than T_1 in the corresponding liquid suspension. These observations suggest that a limited amount of water may become associated with the dried bacteriophage and raise the question of whether this water increases damage to the DNA or to the protein components of T_1. Assays are available for the separate components of the virus system.

Thus, under any conditions of radiation, damage to either the DNA component or the protein component can be assayed. The procedure for the isolation of phage containing 5 bromouracil in their DNA has been developed; these BU phage will provide an independent method for assessing the significance of DNA damage in reducing the plaque forming ability of T_1. It is hoped that the ratios of protein damage to DNA damage for the different conditions of irradiation can be determined; these ratios should give some insight into the mechanisms of direct and indirect action of radiation produced damage.

Repair processes in bacteria: This work is being pursued with Dr. J. Rudé, a Postdoctoral Scholar. Several mechanisms have been characterized for repair of ultraviolet radiation induced damage to bacterial cells. However, in some systems the growth medium of the cells before and after irradiation can have a pronounced effect on the survival of irradiated cells while not affecting the survival of unirradiated cells. The predominant recovery mechanism in Escherichia coli strain B/r is excision-repair. We are designing experiments to determine the reason for the effects of the medium on the efficiency of this repair process. Procedures have been developed for labelling the DNA of E. coli B/r with ^3H thymine. However, it has not been possible to remove entirely acid soluble ^3H even using several "chases" of cold thymine. Since it is essential that the cells be growing in well defined conditions at the time of radiation the procedures to be used to remove acid soluble ^3H are limited. In order to measure excised thymine dimers (the ultraviolet radiation product) and concomitant nucleotides, chemical procedures have been devised for the separation of the various products. Thus, the rate of excision of thymine dimers after irradiation will be measured carefully. Also, the ratio of thymine dimers to other nucleotides will be measured. These two measurements should throw light on the growth medium dependent repair of cells.

Work with Dr. J. Hüttermann, a Postdoctoral Scholar, is reported by Dr. L. S. Myers, Jr., in the Chemical Radiobiology Section.

15. EXPECTED RESULTS IN FY 1972

Research in the areas indicated in (14) will continue. The objectives which will be pursued are:

1. Identification of the monomer product of single strand breaks.

2. Rates of decay of labile phosphate esters (produced by irradiation of deoxynucleotides) determined as a function of pH and temperature.

3. Correlation of findings in the monomer system with those in DNA.

4. Definition of the mechanism of the hydration effect in phage T_1 and attempts to distinguish "direct" and "indirect" effects.

5. Correlation of rate of thymine dimer excision with the medium dependent repair systems in bacteria.

16. EXPECTED RESULTS IN FY 1973

The molecular mechanism by which radiation produces single strand breaks should be well defined. Investigation of the consequences of this mechanism should enable a sensitive assay of radiation damage to DNA to be developed. Use of phage and cellular systems will be made to this end. Other types of DNA damage, i.e. to bases, will be investigated to develop a method for measuring repair of radiation induced base damage in 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: PHYSICAL RADIOBIOLOGY

3. AEC Budget Activity No.:	4. Date Prepared:
06-01-02	April - 1971

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

7. Person in Charge:	8. Project Term:
E. Hardin Strickland	From: 1963 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	3	3	3
(b) Other Tech.	½	1	1
TOTAL:	3 ½	4	4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 35,700	$ 44,300	$ 47,300
(b) Materials and Services	9,000	9,300	10,300
Sub-Total Direct Project Support	$ 44,700	$ 53,600	$ 57,600
(c) Indirect Expenses *	24,900	32,800	34,000
TOTALS:	$ 69,600	$ 86,400	$ 91,600

* 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 1971

Strickland, E. Hardin, Meir Wilchek, Joseph Horwitz, and Carolyn Billups: Low Temperature Circular Dichroism of Tyrosyl and Tryptophanyl Diketopiperazines. J. Biol. Chem. 245, 4168-4177 (1970).

Strickland, E. Hardin, Joseph Horwitz, and Carolyn Billups: Near-Ultraviolet Absorption Bands of Tryptophan. Studies Using Indole and 3-Methylindole as Models. Biochemistry 9, 4914-4921 (1970).

Fretto, Larry, and E. H. Strickland: Effect of Temperature Upon the Conformations of Carboxypeptidase A (Anson), A_{γ}^{Leu}, A_{γ}^{Val}, and $A_{\alpha+\beta}$. Biochem. Biophys. Acta. (Accepted).

Fretto, Larry, and E. H. Strickland: Use of Circular Dichroism to Study the Interactions of Carboxypeptidase A (Anson) and $A_{\alpha+\beta}$ with Substrates and Inhibitors. Biochem. Biophys. Acta. (Accepted).

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 conformational changes induced by ionizing radiation. Preliminary experiments suggest that low doses of radiation may alter the conformation of macromolecules, e.g., DNA, ribonuclease, and lysozyme. A complete analysis of CD and absorption spectra may permit determining which parts of the molecule are involved in conformational 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.

13. RELATIONSHIP TO OTHER PROJECTS

The effects of ionizing radiation on proteins have been investigated by numerous workers. In particular, some aspects of radiation damage of lysozyme have been studied by Aldrich and Cundall in England. 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. Robert Woody (Arizona State), and by Prof. Thomas Hooker (University of California at Santa Barbara).

14. TECHNICAL PROGRESS IN FY 1971

Progress has been made on 4 different aspects of this project: (A) identification of the CD and absorption properties characteristics of the aromatic amino acid moieties, (B) understanding the types of interactions giving rise to strong aromatic CD bands of proteins, (C) interpretation of the CD spectra of proteins, and (D) the effects of Co^{60}-gamma irradiation upon proteins. Each of these areas is described in more detail below.

A. Spectral Properties of Aromatic Amino Acid Moieties. Previously we have described how to identify the tyrosyl and phenylalanyl CD bands of proteins. The tryptophanyl CD bands, however, tend to dominate the near-ultraviolet CD bands of many proteins. During the past year we have completed characterizing the wavelength positions and vibronic spacings of the tryptophanyl absorption bands. This information was obtained by studying the tryptophanyl chromophore (3-methylindole is a model) in nonpolar solvents and by examining the 77°K spectra of proteins containing a single tryptophan residue. The prominent ^1L_b bands of the indolyl chromophore are the 0-0, 0+730 and 0+980 cm^{-1} bands; for ^1L_a electronic transition, the 0-0 and 0+850 cm^{-1} bands can be resolved under favorable circumstances in proteins at 77°K. The 0-0 ^1L_a band usually occurs at longer wavelengths than the 0-0 ^1L_b band in proteins. The position of the ^1L_a band (290-302 nm) is extremely sensitive to the local environment of the indolyl side chain.

B. Origin of CD Bands. The types of interactions giving rise to the aromatic CD bands of proteins were investigated experimentally by using 11 cyclic dipeptides containing a tyrosine or tryptophan residue. The CD intensities are enhanced greatly in those diketopiperazines having two aromatic residues with the L configuration. This enhancement seems to result from coupling of the ^1L_b electronic transition in either a tyrosyl or tryptophanyl moiety with the far ultraviolet transitions in the second aromatic side chain. The effects of conformational motility were examined by cooling the diketopiperazines. Although these compounds have a large CD intensity at room temperature, their increase in intensity upon cooling is much less than for the non-cyclic, monomeric tyrosine and tryptophan derivatives. This finding confirms that the diketopiperazines have fewer conformers at room temperature than do the noncyclic derivatives. For diketopiperazines containing only a single aromatic residue, the conformer with the aromatic side chain folded over the diketopiperazine ring is dominant even at room temperature. In diketopiperazines having 2 aromatic residues with the L configuration, the most stable conformer has both aromatic side chains sharing the space over the diketopiperazine ring. These results suggest that in many proteins the intensities of the tyrosyl and tryptophanyl CD bands may be determined primarily by the motility of the side chains and by the nearness of other aromatic residues. These findings now permit examining the effects of various agents upon the conformations of the tyrosyl and tryptophanyl side chains in proteins.

C. Protein CD Bands. Detailed CD studies have been carried out on carboxypeptidase A. The binding of a number of substrates and inhibitors can be followed by alterations in the near-ultraviolet CD bands of carboxypeptidase A. These CD alterations, when correlated with the X-ray data available for

this enzyme, can be used to identify both primary and auxiliary binding sites for these molecules. The addition of the inhibitor β-phenylpropionate to carboxypeptidase A alters the CD spectrum in the wavelength range where tyrosyl bands occur. Analysis of these data suggests that this change arises primarily from the interaction of β-phenylpropionate with Tyr 198. The substrate Gly-L-Phe does not produce any major alterations in the tyrosyl CD bands of carboxypeptidase A. Apparently the movement of Tyr 248 (known from X-ray studies) into the active site does not cause any readily measureable CD alteration. The binding of Gly-L-Phe, Gly-D-Phe and a number of other compounds containing a phenyl ring shifts the wavelength positions of the tryptophanyl CD fine structure observed in carboxypeptidase A at -196°. This effect may result from binding outside the active site pocket, possibly in the groove near Arg 71.

D. Radiation Studies. Exploratory studies have shown that radiation induced changes in the conformation of the tryptophanyl side chain may be examined using circular dichroism. Irradiation of tryptophan solutions showed that near ultraviolet absorption of the indolyl chromophore is destroyed when attacked by hydroxyl free radicals in the presence of oxygen. In contrast, irradiation of tyrosine solution under the same conditions leads to an increase in the near-ultraviolet absorption, apparently due to the formation of dopa. CD alterations are very difficult to interpret when the irradiation product has an enhanced absorption, because it can increase the CD intensity even though the side chain has become more motile. Consequently, attention has been focused upon examining the effects of Co^{60}-gamma irradiation upon the tryptophanyl side chains of proteins. Lysozyme appears to be the most promising candidate for being able to correlate the CD alterations with the underlying conformational changes. The tryptophanyl CD bands of lysozyme (aqueous solution in air) are appreciably diminished by as little as 12,000 rads. Some loss of intensity of the far-ultraviolet CD bands is also noticed at this dose. CD measurements have shown that irradiation prevents the binding of the competitive inhibitor N-acetyl-D-glucosamine to lysozyme.

15. EXPECTED RESULTS IN FY 1972

Our past work on near-ultraviolet CD bands has progressed to the point where the origin of these bands may be identified in some proteins whose static conformations are known in the crystalline state. The reliability of these identifications will be checked in two ways. First, in view of our previous experimental findings with model compounds, theoretical calculations will be carried out to determine the near-ultraviolet CD intensity resulting from interactions between neighboring aromatic amino acid residues in these proteins. Secondly, the CD spectra of selectively modified proteins will be examined. (Contrary to the impression given in many articles published by others, the CD spectra of chemically modified proteins can only be interpreted reliably after analyzing whether the modified residues may have CD bands and whether their transitions may interact with the unmodified portion of the protein.) These results should permit using the near-ultraviolet CD spectra to identify the dynamic conformation changes occurring in certain proteins. Special attention will be given to interpreting the near-ultraviolet CD spectrum of lysozyme, since it is altered following irradiation. An attempt will be made to identify precisely the effect of gamma irradiation upon the conformation of lysozyme. Especially we shall explore what prevents irradiated lysozyme from binding N-Acetyl-D-glucosamine.

Work will be continued to examine possible conformational changes induced in enzymes during their interactions with substrates and inhibitors. Where

possible, theoretical calculations will be made to facilitate interpreting the CD alterations observed. CD studies will be initiated on the allosteric transitions in enzymes showing feedback inhibition.

16. EXPECTED RESULTS IN FY 1973

Investigations will continue generally along the lines proposed for FY 1972. CD will be used to investigate the effects of irradiation upon enzymes showing feedback inhibition. In addition, gamma irradiation of solutions will be used to probe the exposure of tryptophan residues in a variety of proteins. Other studies will focuse upon identifying the origin of cofactor CD bands in proteins and upon using these bands for studying conformational changes in proteins.

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 - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	3	3	3
(b) Other Tech.	-	-	-
TOTAL:	3	3	3

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 45,600	$ 46,600	$ 49,600
(b) Materials and Services	11,400	10,400	11,700
Sub-Total Direct Project Support	$ 57,000	$ 57,000	$ 61,300
(c) Indirect Expenses *	33,200	32,800	34,000
TOTALS:	$ 90,200	$ 89,800	$ 95,300

* 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 1971

Nishita, H., Haug, R. M., Hamilton, M., and Alexander, G. V. Influence of Soil Heating on the Growth and Elemental Composition of Plants. Soil Sci 110: 61-70. 1970

Nishita, H., and Hamilton, M. Spurious Thermoluminescence of Soils. Soil Sci. 110: 371-378. 1970

Nishita, H., and Hamilton, M. Influence of Temperature During the Gamma Irradiation Period on the Subsequent Themoluminescence of Soils and LiF and CaF_2 Dosimeters. Soil Sci. 111: In press. 1971

12. SCOPE OF THE PROJECT

The general 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 interrelationships problems from the point of view of fundamental mechanisms.

Currently, this Section is involved in several laboratory and field projects.

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

(2) Influence of soil organic matter on Cs137 uptake by plants.

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

- (a) Thermoluminescence of soil fractions and minerals.
- (b) Decay characteristics of soil thermoluminescence.
- (c) Methods of using soil as radiation dosimeter.

(4) Availability of Sr90 and Cs137 and other elements (essential and nonessential) to plants grown in soils exposed to heat and ionizing radiation.

The scope and the objective of these projects are discussed below in the "EXPECTED RESULTS" section. Experiments at the laboratory are conducted in the greenhouse and in plant growth chambers. Several experiments on soil thermoluminescence are being conducted at the Cs137 radiation field in Rock Valley, Nevada Test Site. If and when nuclear excavation studies are opened again under the Plowshare Program, several field experiments on soil thermoluminescence will be conducted.

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:

Biology Department, Battelle - Northwest, Richland, Washington
University of California, Berkeley, California
University of Tennessee, Knoxville, Tennessee
Ecology Section, Oak Ridge National Laboratory, Oak Ridge, Tenn.

14. TECHNICAL PROGRESS IN FY 1971

Plant growth phase of the experiment to study the effect of clinoptilolite on the Sr90 and Cs137 release from soils as a function of time and repeated cropping was completed. Radioassay and the elemental analysis phase is in progress.

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. Chemical analysis of the plant samples are in progress.

Under soil thermoluminescence studies, an experiment to study the thermoluminescence in relation to soil particle size was done. The greatest thermoluminescence was exhibited by either the 88-105 or the 105-125 micron fractions depending on the soil. This occurred with unirradiated fraction (natural thermoluminescence) as well as the irradiated fraction. In measuring thermoluminescence, somewhat better reproducibility was obtained by using certain soil fraction rather than unfractionated soil. In several soils, the large particle size fractions (500-833 and 833-1,000 micron size) showed a much more rapid thermoluminescent decay than the other size fractions. This perhaps reflected the difference of the mineralogical composition among the particle size fractions.

"Natural" thermoluminescence of 36 different samples of minerals was examined. The glow peaks of the minerals occurred in the 250° to 300° C and the 340° to 400° C temperature ranges. The montmorillonitic and the illitic group of minerals tended to show glow peaks at a little lower temperature than the other minerals (kaolinite, halloysite, attapulgite, and quartz). The threshold temperature for the "natural" thermoluminescence of most minerals was around 200°C.

The influence of temperature (0° to 70° C) during the exposure period to Co60-gamma radiation on the thermoluminescence of two soils and LiF (TLD-100) and CaF_2 (TLD-200) dosimeters was examined. The thermoluminescence of these materials were affected by their temperature during irradiation. The effect of temperature during irradiation was eliminated in the soils by using a pre-readout, post-irradiation heating regimen of 100° C for 20 min. or by allowing the thermoluminescent decay to progress for an appropriate length of time. For the two soils examined, this time was about 58 and 168 hr. The pre-readout, post-irradiation heating regimen (100° C for 10 min.) sometimes used for TLD-100 and TLD-200 dosimeters did not eliminate the effect of temperature during irradiation.

15. EXPECTED RESULTS IN FY 1972

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

on contaminated soils.

The objectives of this project were to determine the effect of soil surface application of clinoptiolite on the Cs137 and Sr90 uptake by plants and the effect of clinoptilolite on the release of Sr90 and Cs137 to plants as a function of time and repeated cropping. Analytical phase of the last experiment is in progress. It is anticipated that his project will be terminated during this fiscal year.

(2) Influence of soil organic matter on Cs137 uptake by plants.

The objective of this experiment was to determine the influence of soil organic matter on Cs137 uptake by plants. Soils with added organic matter were incubated in moist condition for various lengths of time up to 17 weeks and cropped by a modified Neubauer technique. Chemical analysis of plant tissue is in progress. This project will be terminated during this fiscal year.

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

(a) 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. As noted above, part of this work has been completed. In the previous experiment, soil fractionation was done before irradiation. In the next experiment, the soil fractionation will be done after irradiation.

(b) Decay characteristics of soil thermoluminescence .
The objective of this study is to determine the decay properties of wide range of different soil types.

(c) Method of using soil as radiation dosimeter.
The objective of this work is to develop several procedures for using soil as a radiation dosimeter and test them for their applicability.

(4) Availability of Sr90 and Cs137 and other elements (essential and nonessential) to plants grown in soils exposed to heat and ionizing radiation.

The soil in the vicinity of a nuclear detonation may be exposed to a wide variation of heat ranging from temperature well above soil fusion down to ambient temperature with increasing distance from ground zero. The objective of this project is to determine the change of plant availability of the radioactive, as well as the stable, nuclides in the soil. One report on this project has been published (Soil Sci. 110:61-70, 1970). The second manuscript on the water soluble and exchangeable Ca, Si, Mg, K, and Na as a result of soil heating is in preparation. An experiment is in progress to determine the release of various elements to plants grown in photon irradiated soils.

16. EXPECTED RESULTS IN FY 1973

Certain aspects of the projects in progress during FY 1972 will be continued. It is anticipated that the soil thermoluminescence studies will

be expanded to determine the effects of irradiating the soils with protons, deuterons, tritons and alpha particles. The recent acquisition of a new cyclotron by this Laboratory makes these studies feasible. If the proper equipment can be mustered, the dependency of soil thermoluminescence on the energy of various ionizing radiation will be initated. This may provide important information in using soil as a radiation dosimeter. Another project on soil-plant interrelationship of Sr90 and Cs137 is being considered presently. It is also possible that this Section will be involved in studying certain aspects of soil nitrogen 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.:	4. Date Prepared:
06-02-04	April - 1971

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:
Evan M. Romney	From: 1953 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	3	2 3/4	3
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3 1/4	3	3 1/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 46,700	$ 44,600	$ 49,600
(b) Materials and Services	13,000	10,400	12,200
Sub-Total Direct Project Support	$ 59,700	$ 55,000	$ 61,800
(c) Indirect Expenses *	33,200	32,800	34,000
TOTALS:	$ 92,900	$ 87,800	$ 95,800

* 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 1971

1. Effect of the Phosphorus Level on the Micronutrient Content of Franseria dumosa. Phyton 26: 151-154, 1969. (with A. Wallace and R. T. Muller).

2. Zinc Sources on Micronutrient Contents of Golden Cross Bantam Corn. Soil Science 109:66-67, 1970. (with A. Wallace).

3. Soil Temperature Effects on Growth of Seedlings of Some Shrub Species which Grow in the Transitional Area Between the Mojave and Great Basin Deserts. Bioscience 20: 158-159, 1970. (with A. Wallace and R. T. Ashcroft).

4. Persistence of Plutonium in Soil, Plants and small Mammals. Health Physics 19: 487-491, 1970. (with H. M. Mork and K. H. Larson).

5. Radiation Doses to Vegetation from Close-in Fallout at Project Schooner. Brookhaven Symposia Series: "Survival of Food Crops and Livestock in the Event of Nuclear War", 1970. (with W. A. Rhoads, H. L. Ragsdale and R. B. Platt).

6. Effects of Chronic Exposure to Gamma Radiation of the Ephedra nevadensis. Radiation Botany (in press) (with H. W. Kaaz and A. Wallace).

7. Some Interactions of Ca, Sr and Ba in Plants. Agron. Jour. (in press) (with A. Wallace)

12. SCOPE OF THE PROJECT

The research objectives of the Plant Factors Section are three-fold in scope: (1) study the effects of radiation from radioactive fallout and from fixed gamma ray sources on vegetation of the desert ecosystem, (2) improve understanding of the function of plants in the biological cycling of radioactive isotopes contaminating the environment, and (3) study the ecological attributes of desert vegetation under natural and manipulated environmental conditions with emphasis on the perennial shrub species. The accomplishment of these objectives involves continued observations of the status of vegetation in fallout contaminated areas and periodic assessments of the cycling of biologically significant radionuclides and mineral elements. Also involved are studies of the influence of abiotic factors (edaphic and climatic) on the propagation and survival of given shrub species and on their subsequent associations and spacial distributions in the desert ecosystem.

Research activities of this Section are part of a team program involving a systems analysis approach to an understanding of the ecological attributes and functions of plants in the desert environment. Investigations are conducted in the field and in the laboratory in order to bridge the gap between practical and theoretical problems arising from radioactive fallout contamination and to help increase understanding of the functional and homeostatic factors involved in maintaining the integrity of desert vegetation.

Our research findings should help establish parameters for assessing the

consequences to man (and to other living components of the environment) resulting from radioactive contamination of the environment, and increase knowledge of certain physiological characteristics and peculiarities of desert plants. Much of the information derived from this project should be directly applicable to the interests and needs of the Nevada Applied Ecology Group, Office of Effects Evaluation, NVOO, and of the U.S./I.B.P. Desert Biome Program.

13. RELATIONSHIP TO OTHER PROJECTS

Research activities are correlated with the work of other investigators in the Environmental Radiation Division, viz. A. Wallace (480344), S.A. Bamberg (480353), V.Q. Hale (480346), O.R. Lunt (480345), H. Nishita (480310), F.B. Turner (480343), and R.A. Wood (480351). Related studies are being conducted at the Biology Department, Battelle-Northwest Laboratory; Radiation Ecology Section, Oak Ridge National Laboratory; Biology Department, Brookhaven National Laboratory; Biology Department Emory University; Biology Division, Lawrence Radiation Laboratory; E.G.and G., Santa Barbara; I.B.P. Desert Biome Program, Logan, Utah; Sections H-4 and H-7, Los Alamos Scientific Laboratory; and Southwest Radiological Health Laboratories, Las Vegas.

14. TECHNICAL PROGRESS IN FY 1971

Work in collaboration with A. Wallace and others continued in the fallout areas of three Plowshare excavation tests at the Nevada Test Site to document the recovery and succession of vegetation. Mixed grasses continued to dominate those areas destroyed by fallout irradiation. New seedling germination and survival has been sufficient to assure eventual recovery of the areas initially dominated by the sagebrush (Artemisia species). However, the new seedling population suffered heavy damage from grazing rabbits during 1970, presumably as the result of the drouth conditions and subsequently poor production of browse. Striking results of the devastating effects of beta irradiation are evident in areas of the Schooner fallout pattern where plots of vegetation were covered with polyethylene plastic for protection from fallout debris. These protected plots of vegetations are thriving and lush from the additional moisture available from the surrounding devastated area. We see the same pattern of succession beginning with reseeding of grasses in the close in fallout pattern from Schooner as has occurred previously in the Sedan, Palanquin and Cabriolet fallout areas. We are paying particular attention to germination and survival of seedlings of native shrub species and their subsequent response to climatic conditions and animal predation. During the past five years of observation we have seen greater evidence of seedling survival in the higher elevation, Great Basin Desert areas of the Nevada Test Site than in the lower elevation, Mojave Desert areas. We have initiated studies to investigate artifical revegetation of some of the disturbed areas resulting from nuclear testing activities. We have successfully developed artificial propagation techniques which could supply ample seedling stock of most of the dominant shrub species common to the Nevada Test Site. We shall continue to investigate husbandry techniques at selected revegetation study sites. The lack of favorable recovery of native shrubs at sites disturbed more than 20 years ago in the Mojave desert areas is convincing of the inability of this system to recover within a reasonable period without mans help.

Work in the environmental study plots at Mercury and Rock Valley continued to investigate the effects of irrigation and nitrogen and phosphorus fertilization on primary productivity. We have developed a systems analysis method of measuring species association between pairs of perennial plants and also a non-destructive dimensional analysis method for determining the above-ground biomass and new season productivity of desert vegetation. Measurements of plant populations are continuing with these methods as part of the documentation work in the I.B.P. Desert Biome validation study site established in Rock Valley. We have added a fourth year of conventional phenological observations correlated with rainfall, soil temperature and soil moisture measurements to data collected from environmental study sites in Frenchman Flat, Mercury Valley and Rock Valley.

Field classification and chemical and physical characterization of soil from 57 sites in the Mojave Desert portion of the Nevada Test Site has almost been completed in collaboration with V.Q. Hale. Chemical analysis of the vegetation growing at these 57 sites has been completed. All data from our field studies are being stored in computer accessible form for use in the systems analysis programs directed toward better understanding of the ecological attributes and functions of plants in the desert ecosystem.

In our controlled laborabory facilities we have continued studies of the mineral nutritional characteristics of prominent shrub species and of the sensitivity of these shrub species to gamma radiation. We have developed successful methods for vegetative reproduction of some 42 different perennial shrubs and herbaceous species common to the Nevada Test Site. Mineral analysis of plant samples from our field and laboratory studies has continued and emphasis has been placed this year on nitrogen determinations. We now have stored into our computer system from the emission spectrograph the concentration levels for 20 elements in more than 19,000 plant samples from field and laboratory studies.

A major effort has been made during this fiscal year to synthesize findings from our laboratory and field studies into reports. In addition to the preparation and submission to journals of several research papers, we have assembled a descriptive monograph exceeding 400 pages on findings from our research projects initiated since 1965.

15. EXPECTED RESULTS IN FY 1972

We shall continue to document the recovery and succession of vegetation in areas damaged by radioactive fallout. Another radiological survey will be conducted at our persistence study sites in off-site fallout study areas as part of our continuing 5-year interval spot check on the cycling of biologically significant radionuclides. Additional studies will be initiated in plutonium contaminated areas in order to further investigate the persistence of Pu in soil, plants, and small mammals. We shall continue to investigate practical methods for the revegetation of areas denuded from nuclear testing and other activities at the Nevada Test Site.

Work at our environmental study sites will continue to evaluate the effects of certain edaphic and climatic factors on primary productivity. Assessments of plant populations shall include species associations,

spacial distributions and biomass determinations. An intensive analytical program will be continued in conjunction with the soil survey of study areas at the Nevada Test Site. This will include assessments of radionuclides in soil.

Laboratory studies at UCLA will be continued to support and confirm findings from field studies with emphasis being placed on further evaluations of the mineral nutritional and physiological characteristics and peculiarities of desert plants. Reports from completed phases of our research will be prepared and submitted for publication. This will include another large descriptive monograph on soil and plant relationships.

16. EXPECTED RESULTS IN FY 1973

We plan to continue all phases of field and laboratory work in progress. New studies will be initiated as certain phases of the work reach completion in further pursuit of our research objectives to gain further understanding of the radiology, ecology and physiology of desert plants. We expect to have made marked progress in our systems analysis approach to an understanding of the ecological attributes and functions of plants in the desert 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: ENVIRONMENTAL FACTORS

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

4. Date Prepared: April - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	3	2 3/4	3
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3 1/4	3	3 1/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 38,800	$ 37,600	$ 41,600
(b) Materials and Services	10,800	9,300	10,800
Sub-Total Direct Project Support	$ 49,600	$ 46,900	$ 52,400
(c) Indirect Expenses *	24,900	24,600	25,500
TOTALS:	$ 74,500	$ 71,500	$ 77,900

* 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 IN FY 1970

None

12. SCOPE OF THE PROJECT

We are approaching a systems analysis treatment of the transit of long-lived radionuclides (Sr90, Cs137) through a dairy enterprise which marketed more than half the milk consumed in St. George, Utah from local processing plants. The data bank for feeding trials is completed, and a limited-scope descriptive model is under construction for this part of the farm system. A system study requires that all data be present.

This is an important program. Our thesis is one of investigating under "simultaneity" those variables that control transfer of the elements of concern. The crash programs associated with the testing of nuclear weapons often generated relatively little information. In retrospect, the main obstacles were lack of base lines from which to evaluate observations made on specimens taken at field stations after deposition of fallout. Consequently, the effort frequently degenerated into simply monitoring extant radioactivity levels, the information became "single-factor" and limited in biologic contribution, though important for legal or liability purposes.

Incorporation of stable element congeners of the radionuclides into the interpretations projects two complementary time scales upon the study. "Acute" contamination conditions are provided by the radionuclides in surface contamination and "chronic" contamination conditions are found from root uptake by plants, simulating equilibrium distribution in root zones.

13. RELATIONSHIP TO OTHER PROJECTS

Soil moisture and soil temperatures are recorded in a collaborative study with Ecology of the Nevada Test Site Section (Dr. Janice Beatley), at NTS, upon 68 sites chosen for edaphic and environmental diversity. Subjects of mutual interest are explored with Soil Factors Section (Dr. H. Nishita) in growth chamber and laboratory experiments. A limited statistical collaboration with Battelle Northwest Ecosystems Department (Dr. Lee Eberhardt) on frequency functions of the Farm Program is operative.

A new program, initiated in FY 1971, was launched at University of Florida Department of Environmental Engineering (Dr. J. F. Gamble) to deal with transfer of Cs137 in local food chains - beef and milk - leading to consumable products.

14. TECHNICAL PROGRESS IN FY 1971

All our effort was put into the Farm Program. Dairy feeding trial transfer coefficients took 30 man months to finish, 12 being for stable element atomic absorption spectrophotometry for Cs (420 specimens), Sr, Ca, Mg, and Rb (167 determinations each). Supervision was supplied to get 550 specimens ready for radionuclide determinations through wet chemistry and for gamma-Cs137.

15. EXPECTED RESULTS IN FY 1972

The analytical data from Radiochemistry should be assembled into a data bank.

16. EXPECTED RESULTS IN FY 1973

All technically competent manpower will be applied to data interpretation.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: RADIATION ECOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
B. G. Maza (Acting)

8. Project Term:
From: 1959 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2	1 3/4	3
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	2 1/4	2	3 1/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 26,800	$ 26,600	$ 47,600
(b) Materials and Services	9,000	6,900	10,000
Sub-Total Direct Project Support	$ 35,800	$ 33,500	$ 57,600
(c) Indirect Expenses *	24,900	16,400	34,000
TOTALS:	$ 60,700	$ 49,900	$ 91,600

* 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 1971

Mullen, R. K. The Energy Metabolism of Four Species of Desert Rodents in Their Natural Environments. UCLA #12-805. 1970

Mullen, R. K. Respiratory Metabolism and Body Water Turnover Rates of Perognathus formosus in its Natural Environment. Comp. Biochem. Physiol. 32:259-265. 1970

French, N. R., C. D. Jorgensen, M. H. Smith, and B. G. Maza. Comparison of Some IBP Population Estimation Methods for Small Mammals. Grasslands Mimeographed Report, 25 pp. 1970

12. SCOPE OF THE PROJECT

The primary objective of this section is to evaluate effects of continuing exposure to gamma radiation on populations of small mammals, and attendant changes in the overall structure and behavior of an irradiated natural community.

Demographic parameters (life-span, fertility, and age structure) of irradiated rodent populations are being evaluated in the field, and compared with control populations.

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

13. RELATIONSHIP TO OTHER PROJECTS

In the Rock Valley study area, where our field investigations are conducted, other investigators of this laboratory are studying reptiles, invertebrates, and plants. Demographic analysis of small mammal populations is being conducted at Oak Ridge National Laboratory, Savannah River Operations, and the University of Pittsburgh. Effects of radiation on laboratory colonies of native species of rodents is under investigation at the University of Georgia, Northrup Space Laboratories, Argonne National Laboratory, and Battelle Northwest. The Rock Valley study plots have been integrated with the desert biome studies of the U. S. International Biological Program (Analysis of Ecosystems), and active cooperation with management of both desert and grassland biomes is being carried on.

14. TECHNICAL PROGRESS IN FY 1971

1. During the summer of 1970 the fenced control plots in Rock Valley were used to test the efficiency of several trapping procedures. This work was undertaken in collaboration with personnel of the U.S.I.B.P. grasslands biome (from Colorado State University) and the Savannah River Ecology Laboratory. The results of this study indicated that past procedures in Rock Valley (3 nights of consecutive trapping) successfully enumerate all of the individuals of most of the resident species. For example,

following three nights of trapping in Plot C in mid-July, personnel of the Savannah River Ecology Laboratory trapped for another two weeks in the same area. Yet the extra two weeks of effort registered only three individuals (two of these were diurnal ground squirrels) not trapped during the initial 3-day period:

Species	July 14	July 15	July 16	others between July 17-30	Totals
Perognathus formosus	105	-	-	-	105
P. longimembris	3	-	-	-	3
Dipodomys merriami	8	-	-	-	8
D. microps	2	-	-	-	2
Ammospermophilus leucurus	-	2	-	2	4
Onychomys torridus	10	-	-	1	11
Totals	128	2	-	3	133

2. Food consumption by heteromyid rodents in Rock Valley has been analyzed by identification of material in stomach contents. Ingested material was broken down in terms of leaves, fruits, seeds, flowers, insects, etc. Chi-square tests indicated no differences between four heteromyids in relative utilization of major food categories. However, these analyses did not attempt to discriminate between possible differences in species of plant materials consumed. All four rodent species relied heavily on the grass Bromus rubens and on the cruciferous annual Thelypodium lasiophyllum. Various parts of these two species alone compose about 50% of the diets of the four rodents. Annual differences in relative abundance of certain plant species reflect simple opportunism in the feeding of the rodents. For example, the presence of Thelypodium parts in stomachs (in 1966 and 1967) was roughly proportional to its availability as a food source. In conjunction with these studies of food utilization by rodents, estimates were made of food production in Rock Valley during the years 1966, 1967, and 1968. Striking differences between years were revealed, and these differences are clearly related to varying success in reproductivity of the rodent populations.

Estimated production (kg/ha) of seeds and perennial leaves in three plots in Rock Valley

	A		B		C	
Year	seeds	leaves	seeds	leaves	seeds	leaves
1966	66.3	24.5	105.8	21.5	71.1	26.1
1967	5.8	14.0	5.0	12.9	5.3	15.0
1968	80.9	22.9	58.3	21.5	68.9	23.6

3. Home ranges of heteromyid rodents have been analyzed in terms of mean recapture radii over a period of six years:

Plot	Number of animals	Number of captures	Mean recapture radius (m)
A	340	10,167	18.9
B	567	17,678	18.2
C	345	8,632	22.3
D	195	3,371	17.5

A 3x2x3 factorial analysis of variance involving three fenced plots, sex, and three different years indicated strongly significant differences between males and females and between years, but only slight indication of differences between plots ($F = 3.33$, $F_{.05} = 3.00$). In general, although home range sizes show considerable variation, there is no indication that the irradiation of Plot B has had a measurable effect on this parameter.

15. EXPECTED RESULTS IN FY 1972

1. Work will continue on the analysis and interpretation of data acquired in Rock Valley between 1962 and 1968: survivorship and fertility of rodents, the correlation of external morphological characteristics and reproductive state in heteromyids, age determination in heteromyids, and the correlation of reproductive success with various environmental and biotic variables.

2. The irradiation experiment in Rock Valley will continue, in order to determine whether the irradiated mammals persist in the face of continuing radiation stress while some other vertebrates are becoming extinct.

3. The densities and age-distributions of mammal populations occupying the I.B.P. validation site will be periodically assessed, and this work expanded to include diurnal species as well as larger carnivores.

16. EXPECTED RESULTS IN FY 1973

Results in FY 1973 will depend upon the new investigator. Work with Rock Valley mammals in connection with the irradiation experiment will continue, with particular emphasis on possible indirectly induced changes. Investigations on the I.B.P. validation site will continue.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: ECOLOGY OF THE NEVADA TEST SITE

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

4. Date Prepared: April - 1971

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
Janice C. Beatley

8. Project Term:
From: 1962 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	3	2 1/4	2 1/2
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3 1/4	2 1/2	2 3/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 34,500	$ 29,600	$ 29,600
(b) Materials and Services	10,300	7,100	8,200
Sub-Total Direct Project Support	$ 44,800	$ 36,700	$ 37,800
(c) Indirect Expenses *	24,900	24,600	17,000
TOTALS:	$ 69,700	$ 61,300	$ 54,800

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

11. PUBLICATIONS DURING FY 1971

Beatley, Janice C. Perennation in Astragalus lentiginosus and Tridens pulchellus in relation to rainfall. Madrono 20: 326-332. 1970.

Beatley, Janice C. Ecologic and geographic distributions of the vascular plants of southern Nye County, and adjacent parts of Clark, Lincoln, and Esmeralda Counties, Nevada. UCLA 12-800 (Supplement to UCLA 12-705), Laboratory of Nuclear Medicine and Radiation Biology, Univ. of California, Los Angeles. 49 pp. 1971.

12. SCOPE OF THE PROJECT

Objectives have been the determination of certain environmental and biological baselines on selected sites, representing the major kinds of ecosystems on the Nevada Test Site (exclusive of Pahute Mesa), to enable interpretation of the biological effects of nuclear and other testing. A major ancillary project has been the collection and identification of the vascular plants in relation to plant communities and physiographic areas of southern Nye County, Nevada.

13. RELATIONSHIP TO OTHER PROJECTS

Ecologic, floristic-faunistic, and environmental studies conducted elsewhere in desert regions, and radiation effects studies on other AEC-DBM contracts, have more or less pertinence to the total program.

14. TECHNICAL PROGRESS IN FY 1971

Ecology studies. Collection of environmental measurements data has continued on the 68 permanent study plots, for continuing characterization of the environment of each of the sites and the environmental mosaic of the Test Site. Certain plant data were collected on certain of the sites to meet particular needs for additional information.

Vascular Plants of Southern Nye County. An additional 2000 plant collections were accessioned, including around 200 new taxa, bringing the total to nearly 1100 taxa now known from the region. Approximately 4500 duplicates were distributed to monographers and institutional herbaria over the country, and several more new species are being described for the literature. Because of the absence or near absence of herbaceous species in southern Nevada in the spring of 1970, decision was made to delay until after the spring of 1971 the preparation of the final publication.

15. EXPECTED RESULTS IN FY 1972

Plant collecting and vegetation reconnaissance will continue in selected sampling areas of southern Nye County in the spring of 1971, following which the manuscript for the final publication will be prepared.

Preparation of other manuscripts, dealing with the 6-8 years of environmental and biological data -- in the construction of models for each of the 68 permanent study sites, and for Mojave, transitional, and Great Basin Desert systems -- is contingent upon the availability of a biostatistician, without which the data analyses cannot proceed.

16. EXPECTED RESULTS IN FY 1973

Data analyses and manuscript preparation, contingent upon the services of a professional biostatistician.

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 - 1971

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

6. Working Location: UCLA and NEVADA TEST SITE

7. Person in Charge: Frederick B. Turner

8. Project Term: From: 1961 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	1 ½	2 ½	3
(b) Other Tech.	½	½	½
TOTAL:	2	3	3 ½

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 37,300	$ 42,600	$ 46,600
(b) Materials and Services	9,600	8,600	10,300
Sub-Total Direct Project Support	$ 46,900	$ 51,200	$ 56,900
(c) Indirect Expenses *	24,900	32,800	34,000
TOTALS:	$ 71,800	$ 84,000	$ 90,900

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

11. PUBLICATIONS DURING FY 1971

The demography of the lizard, Uta stansburiana Baird and Girard, in southern Nevada. J. Anim. Ecol. 39: 505-519. (With G. A. Hoddenbach, P. A. Medica, and J. R. Lannom, Jr.)

The ecological efficiency of consumer populations. Ecology 51: 741-742.

Observations of lizards and tree frogs in an irradiated Puerto Rican forest. In: A Tropical Rain Forest. A study of irradiation and ecology at El Verde, Puerto Rico, H. T. Odum, ed., pp. E-25 to E-49. Division of Technical Information, U.S. Atomic Energy Commission. (With C. S. Gist).

12. SCOPE OF THE PROJECT

The effects of continuous exposure to low levels of gamma radiation on natural populations of animals are poorly understood. At acutely sublethal doses, populations may persist for years before succumbing to radiation effects operating through impairment of reproductive or genetic processes. The primary objective of this study is to define the effects of continuous exposure to low levels of gamma radiation on populations of vertebrates. However, the study also considers the impact of chronic irradiation on the natural community as an entity, both in terms of its organization and function.

One feature of this study is to observe the modification of population parameters by irradiation, and to correlate observed changes with dose experience. The Rock Valley program entails the irradiation of natural populations in such a way that all individuals receive approximately equal exposures. However, it is important to evaluate the actual doses sustained by animals as precisely as possible. Tissue doses to individual organisms have been estimated by the use of small thermoluminescent dosimeters.

Studies of annual changes in the reproductive performances of vertebrate populations are being made. The causes of year-to-year differences in reproduction and survival are being investigated in order to clarify the mechanisms governing population size and to yield data necessary to understand the energy dynamics of the community.

Work to data has shown that continued irradiation--at the levels employed in Rock Valley--do alter the demographic function of some species populations. Although no complete extinctions have yet occurred, several species are destined for this fate. The development of a new equilibrium community following selective extinctions, and the time required for such an equilibrium to become manifest, is a continuing feature of the Rock Valley studies.

13. RELATIONSHIP TO OTHER PROJECTS

The two major long-term studies involving irradiated populations of both plants and animals have been those at Brookhaven National Laboratory and at the Puerto Rico Nuclear Center in San Juan. Another study, involving the application of simulated fallout to relatively small experimental plots, has been conducted at Oak Ridge National Laboratory, and a number of studies involving experimental irradiation of plant communities have been conducted at Oak Ridge, the University of Georgia's Savannah River

Ecology Laboratory, and Emory University.

Additionally, work related to one or another portions of the above is being carried out at the:

University of Minnesota, Minneapolis, Minnesota
Colorado State University, Fort Collins, Colorado
Utah State University, Logan, Utah
University of Washington, Seattle, Washington
Lawrence Radiation Laboratory, Livermore, California
University of Nevada, Reno, Nevada
Battelle-Northwest, Richland, Washington
National Reactor Testing Station, Arco, Idaho
University of Michigan, Ann Arbor, Michigan
University of Texas, Austin, Texas

14. TECHNICAL PROGRESS IN FY 1971

1. During this fiscal year a new study area was established in Rock Valley. This plot, henceforth referred to as the "validation site" adjoins the old 20-acre enclosures, and has an area of about 1/2 km^2. During the spring of 1971 measurements of the state of populations of arthropods and vertebrates were made on the validation site as well as in the 20-acre enclosures. Work with insects was made possible by money from the National Science Foundation and by the cooperation of Dr. Elbert Sleeper of Long Beach State College at California. The sampling included the use of pitfall can traps, as was done previously, and the collection of shrub-dwelling species by vacuuming procedures. Three new technicians have been employed in the conduct of this work. Trapping of mammals and reptiles in the new area was carried out during the spring. Counts of breeding birds were made during three two-week periods during the spring. These investigations included both the validation site and the 20-acre enclosures.

2. The development of female sterility among irradiated leopard lizards (Crotaphytus wislizenii) in the irradiated plot has been discussed previously. During 1970 investigations of this problem were extended to the whiptail lizard (Cnemidophorus tigris). As before, some individuals were removed from the irradiated area for study. Of four experimental females examined by laparotomy, three apparently lacked ovaries and had distinctly hypertrophied fat bodies. Three male whiptail lizards from the irradiated plot did not differ in sexual condition from three control males. Experimental administration of follicle-stimulating hormone to the three apparently sterile females and to controls had no effect on the oviducal state of the irradiated lizards but resulted in marked oviducal growth and glandularization among controls. It appears that sterility among female whiptail lizards has developed later and less uniformly than in female leopard lizards, and this difference is consistent with the lower annual radiation doses sustained by most whiptails. Observations during 1970 have also indicated that reproduction among horned lizards (Phrynosoma platyrhinos) has been impaired, and this failure seems attributable to the experimental radiation treatment.

Reproduction by the irradiated population of Uta stansburiana has continued without obvious reduction. The first three species are long-lived types, with sexual maturity normally deferred until the age of 22 months. Uta,

on the other hand, is sexually mature at an age of 8-9 months, and rarely lives longer than 24 months. The longer-lived species are clearly much more vulnerable to sustained and accumlating stresses, such as chronic irradiation. The picture is quite similar to that developed by the University of Wisconsin scientists in their models of the impact of long-continued DDT use on natural communities. Again, the most vulnerable species are the longest lived, and it is these forms which are susceptible to gradual extinction. If our interpretation of the Rock Valley data are correct, and if the pattern of response observed among lizards has bearing on future effects of radiation on other animal populations in Rock Valley, we will witness the gradual disappearance of longer-lived species from the experimental enclosure. The longest-lived species are often predatory types of low density. These forms are not particularly important in terms of energy flow within the community, but they may have important influences upon their respective prey populations. An understanding of such indirect consequences is indispensable for development of predictions of long-term effects of low levels of gamma radiation on ecosystems.

Ages and durations of exposure of female lizards removed from the irradiated 20-acre area in Rock Valley, Nevada

Species	Number	Age when removed from plot (months)	Years exposed to radiation	Year removed from plot	Condition upon removal	Notes on past reproductive condition
Crotaphytus	1112	94	5.4	1969	sterile	yolked follicles in 1965
	1174	82	4.4	"	"	yolked follicles in 1966
	1392	46	3.8	"	"	-
Cnemidophorus	1137	81	5.4	"	"	-
	1149	94	6.4	1970	"	yolked follicles in 1967
	1213	70	5.8	"	"	small yolked follicles in 1969
	1485	46 - 58	3.8 - 4.8	"	fertile	-
	2142	46 - 58	3.8 - 4.8	"	sterile	3 yolked follicles in 1969

3. Possible genetic effects of long-continued irradiation of the lizard, Uta stansburiana, in Rock Valley were examined. We analyzed i) frequencies of alleles controlling selected proteins among lizards occupying the irradiated and two nonirradiated areas, and ii) the genetic variability of the two classes of populations. Samples from the three areas in Rock Valley showed no genetic variation in 10 of the 19 proteins analyzed. Tests of the relative abundance of polymorphic alleles at the 9 variable loci among the three populations revealed no significant difference between areas. Genetic variation of the three populations was estimated in terms of the proportion of polymorphic loci, average individual heterozygosity,

and the number of alleles per locus. There were no apparent differences between the irradiated and nonirradiated populations:

Plot	n	Proportion of loci polymorphic	Average number of alleles per locus	Average individual heterozygosity
A	16	0.37	1.63	0.086
B*	20	0.32	1.68	0.076
D	20	0.37	1.63	0.082

* irradiated

4. Attempts to simulate energy flow in Rock Valley populations of Uta stansburiana were resumed. A new, simpler model relating body temperatures to regimes of light and air temperature was devised. The approach was expanded to include 1966-7 and 1967-8 as well as the year 1965-6. Thus, for a period of three years, we are examining energy flow (respiration as well as yield and changes in standing crop) for a population changing daily in size and composition, and responding to hourly changes in conditions of light and temperature. One purpose of this work will be to experiment with the computer program simulating the energy utilization processes in order to determine the relative importance of various input variables. From these investigations it is hoped to determine what simplifications are most efficacious in modeling energy flow in Uta, and which parameters need to be given the greatest care in measurement.

15. EXPECTED RESULTS IN FY 1972

1. One of the most important aspects of the continuing research in Rock Valley will have to do with changes in the irradiated community, as this system slowly moves towards a new equilibrium imposed by the gradual and selective extinctions of the more sensitive species. We are now able to identify at least one variable determining sensitivity (i.e., life-span), but other parameters are evidently involved. Some of the problems which may presently be foreseen:

a. What indirect effects may be manifested owing to the gradual removal of a large predatory species like the leopard lizard? Are the apparently longer life-spans so far observed among the irradiated Crotaphytus statistically real, and if so, to what degree will this compensatory enhancement of survival delay the disappearance of this species?

b. Has the same pattern of sterility developed among horned lizards? Sampling data indicate that this is probably so, but the situation needs to be assessed by laparatomy if possible.

c. Can a species like Cnemidophorus tigris actually adjust to the radiation-induced sterilization of a portion of the female population and still sustain itself by compensatory reactions among the younger females? Ovarian destruction in Cnemidophorus apparently takes longer to

occur because this species receives lower annual doses of radiation than leopard lizards. Hence, there is always a portion of the population (females less than 3-4 years old) still able to reproduce. Whether this group can carry the population is not yet known, but the possibility cannot yet be eliminated.

d. Why are the heteromyid rodents able to sustain apparently unimpaired reproduction when some of the oldest females in the population have received cumulative radiation doses as large as those known to be sterilizing among leopard lizards?

2. The other major endeavor in Rock Valley, which must be carried on in conjunction with the irradiation experiment, is the periodic assessment of the states of arthropod and vertebrate populations. The acquisition of these "validation" data are important to the successful conduct of modeling efforts by desert biome personnel at Utah State University.

3. 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 investigaror subject enclosed populations to various experimental combinations of physical and biotic factors.

16. EXPECTED RESULTS IN FY 1973

The program in FY 1973 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 meterological 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: PLANT PHYSIOLOGY ECOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
Arthur Wallace

8. Project Term:
From: 1958 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2 ½	1 ½	2
(b) Other Tech.	½	½	½
TOTAL:	3	2	2 ½

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 35,400	$ 28,600	$ 36,600
(b) Materials and Services	15,200	8,800	10,900
Sub-Total Direct Project Support	$ 50,600	$ 37,400	$ 47,500
(c) Indirect Expenses *	24,900	16,400	25,500
TOTALS:	$ 75,500	$ 53,800	$ 73,000

* 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 1971

Wallace, Arthur 1970. Monovalent-ion Carrier Effects on Transport of Rb^{86} and Cs^{137} into Bush Bean Plants. Plant and Soil 32:526-530.

Wallace, Arthur 1970. Water use in a Glasshouse by Salsola kali Grown at Different Soil Temperatures and at Limiting Soil Moisture. Soil Sci. 110(2):146-149.

Wallace, Arthur 1970 Book Review: Ecological Aspects of Mineral Nutrition of Plants. Edited by I. R. Rorison et al. F. A. Davis Co., Philadelphia, Pa. Soil Sci. 110(3):219-220.

Wallace, Arthur, Evan M. Romney, and Rulon T. Ashcroft 1970. Soil Temperature Effects on Growth of Seedlings of Some Shrub Species Which Grow in the Transitional Area Between the Mojave and Great Basin Deserts. Bio Science 20(21):1158-1159.

Abou-Zamzam, A. M. and A. Wallace 1970. Some Characteristics of the Mitochondrial and Soluble Forms of Malate Dehydrogenase in Lemon Fruits. Biochim. Biophys. Acta. 220:396-409.

Wallace, Arthur and Rulon T. Ashcroft 1970. Significance of Vapor in Plant Water Ecomony. Advan. Front. of Plant Sci. 26:153-159.

Wallace, Arthur 1970. Water use in a Glasshouse by Zea mays Grown at Different Soil Temperatures and at Limiting Soil Moisture. Advan. Front. of Plant Sci. 26:145-151.

Wallace, A., R. T. Ashcroft, M. W. M. Leo, and G. A. Wallace 1970. Effect of Cycloheximide, Gamma Irradiation, and Phosphorus Deficiency on Root Pressure Exudation in Tobacco. Plant Physiol. 45:300-303.

Wallace, A., A. M. Abou-Zamzam, and G. A. Wallace Gamma Radiation Effects on ^{22}Na and ^{86}Rb Transport in Bush Bean Plants. Radiation Botany (In press)

Wallace, A., V. Q. Hale, G. E. Kleinkopf, and R. C. Huffaker Carbosydismutase and Phosphoenolpyruvate Carboxylase Activities From Leaves of Some Plant Species From the Northern Mojave and Southern Great Basin Deserts. Ecology

12. SCOPE OF THE PROJECT

The general objectives of the project are to assess the nature of mineral and radionuclide cycling in the ecosystems at the Nevada Test Site and also to determine effects of ionizing radiation on the plant portion of the ecosystem involved.

In collaboration with another section a field plot program is being studied ecologically. Long-time assessments of changes resulting from manipulation are being studied. Mineral cycling studies with the emission spectrograph and neutron activation techniques are part of this project.

Primary productivity, plant phenology, root growth, soil moisture studies, soil-plant relationships including salinity, susceptibility to ionizing

radiation, and plant nutrition are some of the subjects being studied in the field as well as in the laboratory and glasshouse.

The effects on plants of the ^{137}Cs source at Rock Valley continues to be studied and such studies are being intensified with the adjacent establishment of the IBP validation study site.

Analyses of vegetation spacing and patterning is receiving attention both in disturbed and in undisturbed areas. Special attention is being given to the revegetation problem in areas which have been denuded as a result of testing.

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, and by W. A. Rhoads, EG&G, Santa Barbara, California.

14. TECHNICAL PROGRESS IN FY 1971

In cooperation with another section, many analyses of the field plot project were completed and prepared for publication. Analyses of the effects on plants to date of the radiation source in Rock Valley were completed and published. A report was made of the revegetation which has occurred following destruction as a result of nuclear testing. A study of stable cesium cycling in desert ecosystems was undertaken. A study of some of the effects of temperatures on desert plants was completed. Several aspects of ecophysiological studies of desert plants were completed. Analysis of vegetation on a plot established for cooperation with the desert biome IBP was started.

15. EXPECTED RESULTS IN FY 1972

A 300-page monograph on the ecology of soil-plant relationships in desert conditions will be completed early in FY 1972. All phases of the studies

will be continued throughout FY 1972. Mineral and radionuclide cycling studies will be expanded. Soils from contaminated areas will be brought to the glasshouse at the CETO building in Mercury for growing plants for cycling studies.

16. EXPECTED RESULTS IN FY 1973

All aspects of the studies in progress in FY 1972 will be continued in FY 1973. Special emphasis will be on mineral and radionuclide cycling and on revegetation.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: PHYSIOLOGY OF MINERAL ACCUMULATION IN PLANTS

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

7. Person in Charge: O. R. Lunt

8. Project Term: From: 1966 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	1	1	1 1/2
(b) Other Tech.	-	-	-
TOTAL:	1	1	1 1/2

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 15,400	$ 15,600	$ 21,600
(b) Materials and Services	5,500	5,500	6,400
Sub-Total Direct Project Support	$ 20,900	$ 21,100	$ 28,000
(c) Indirect Expenses *	8,300	8,200	17,000
TOTALS:	$ 29,200	$ 29,300	$ 45,000

* 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 1971

None.

12. SCOPE OF THE PROJECT

Soil-water-plant relations: Work continues on in cooperation with Dr. John Letey of UC Riverside on transpirational and photosynthetic activity of Mojave Desert species as a function of soil water potential. 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.

Mineral nutrition studies: A survey on nitrogen fixation at the Nevada Test Site, using the acetylene reduction method, has been completed and the results prepared for publication. A study on calcium accumulation in roots has been completed. The study has been submitted for publication.

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. The soil-water-plant studies are coordinated with studies by Drs. Wallace and Bamberg conducted at this laboratory. Our studies are with plants with confined root systems so that relationships of water potential and net photosynthesis, transpiration, survival, and recovery may be studied. The former studies concern primary productivity under field conditions.

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 1971

The relationship between transpiration, net CO_2 assimilation and soil water potential has been studied in Artemisia tridentata. Data on one run are included which received an irrigation after the 11th day. Water potential dropped to -63 bars in one case and to -72 bars in the other. It is notable from these data that transpiration rates began to decline rapidly after potentials reached about -4 bars. Net CO_2 assimilation began to decline precipitously at slightly higher water potentials, i.e., about -2 bars. Transpirational losses dropped to about zero but not until water potentials were in the range of about -60 bars. The compensation point on net photosynthesis was reached when water potential was in the range of -30 bars. Quantitative data of this nature should contribute importantly to better understanding of factors affecting species distribution, productivity, and survival in the desert. (See attached figures for details).

Under a program partially supported by the NSF it was shown that about 50% of the calcium absorbed by corn roots was non-metabolically accumulated and was believed to be associated with the cell wall. Accumulation into stelar tissue was low and little affected by the presence of the shoot. The corresponding cortical tissue showed a high capacity for accumulation but sustained accumulation requires a continuous supply of photosynthates from the shoot. Strontium behavior in plants is generally similar to that of calcium.

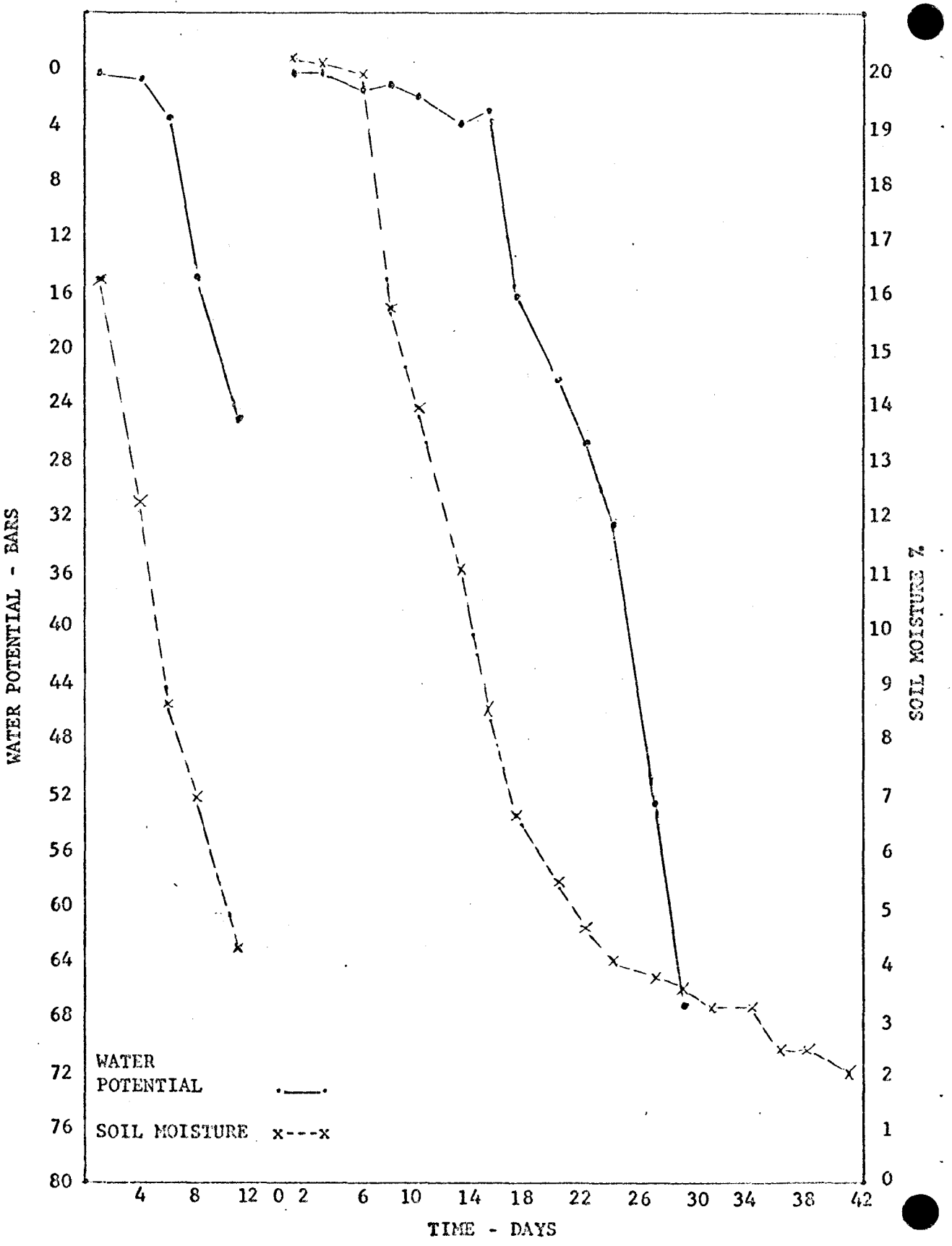
15. EXPECTED RESULTS IN FY 1972

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

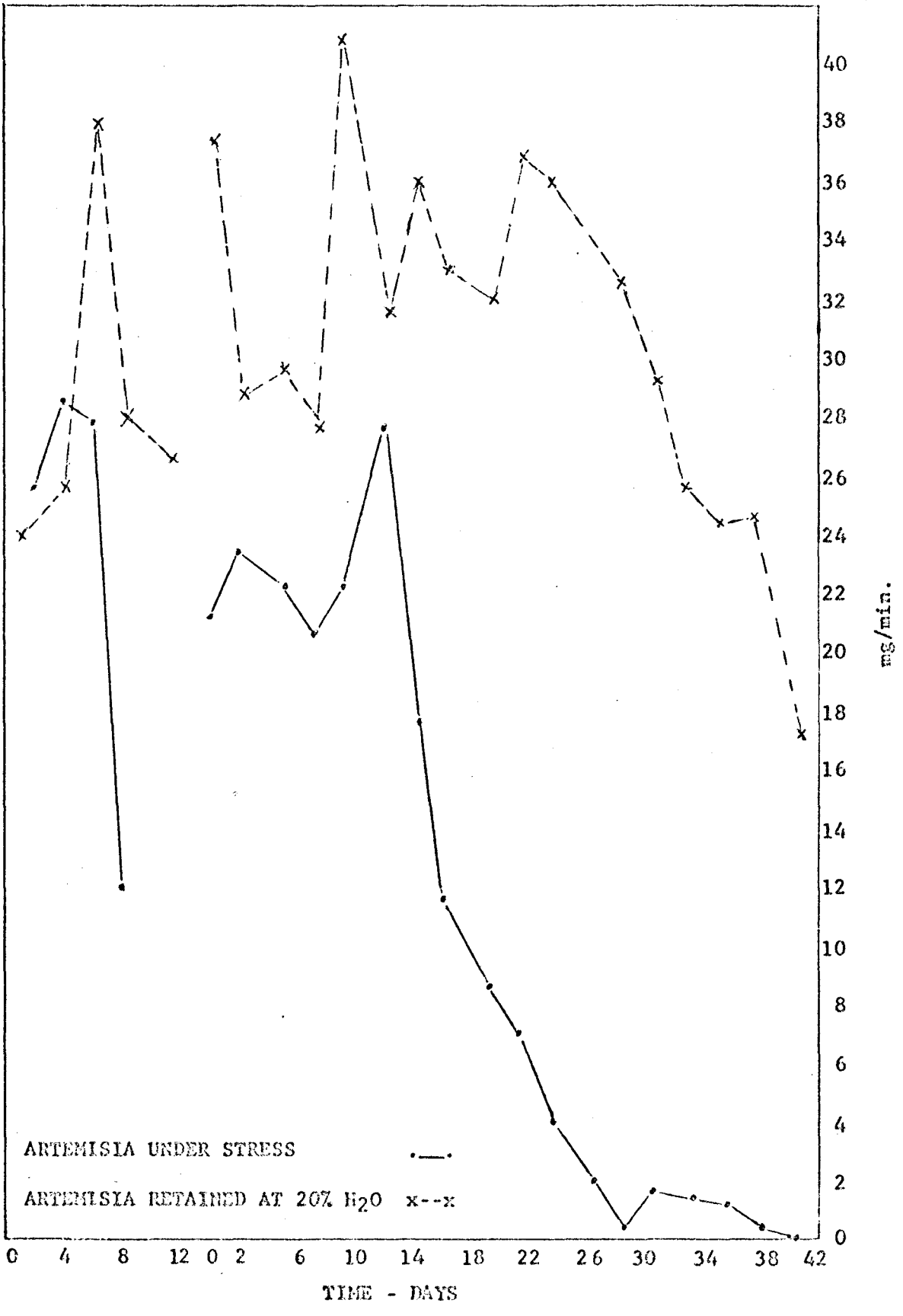
16. EXPECTED RESULTS IN FY 1973

Quantitative data on the response to soil moisture of several desert species: i.e., Atriplex convertifolia, Eurotia lanata, Franseria dumosa, Larrea tridentata, Artemisia tridentata, Festuca octoflora and Chaenactis carphoclinia will be included in the studies now underway. It is expected that in FY 1973 these studies will be sufficiently progressed that correlations of the laboratory data with field observations on distributions, productivity, soil properties, etc., will be undertaken.

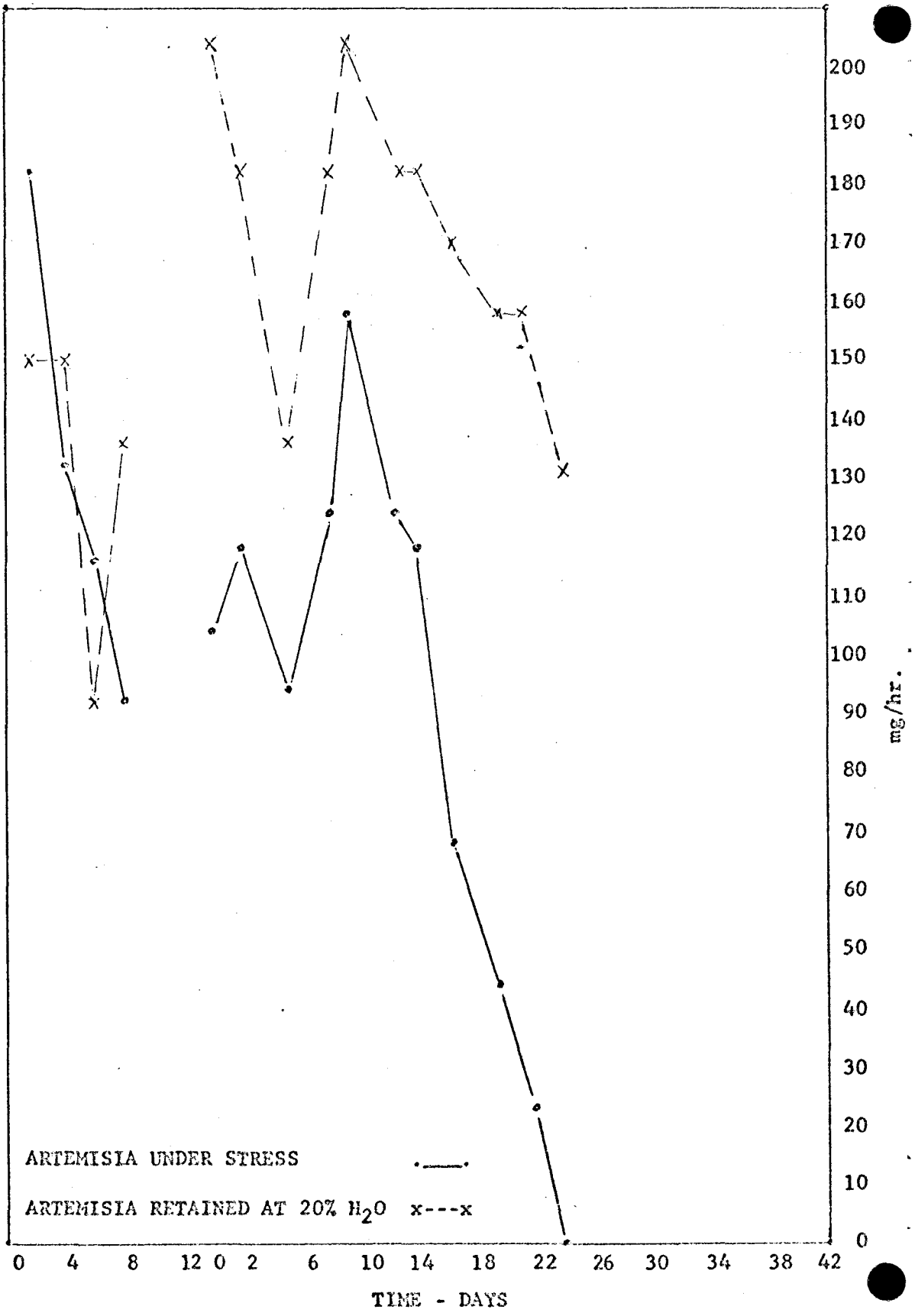
ARTEMISIA TRIDENTATA



TRANSPIRATION LOSSES



NET CO_2 ASSIMILATION



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.:	4. Date Prepared:
06-02-04	April - 1971

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:
Verle Q. Hale	From: 1970 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	1	$\frac{3}{4}$	0
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	0
TOTAL:	$1\frac{1}{4}$	1	0

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 17,200	$ 15,000	$ 0
(b) Materials and Services	5,500	5,200	0
Sub-Total Direct Project Support	$ 22,700	$ 20,200	$ 0
(c) Indirect Expenses *	8,300	8,200	0
TOTALS:	$ 31,000	$ 28,400	$ 0

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

11. PUBLICATIONS DURING FY 1971

Hale, V. Q., Effect of Chelates on Uptake of Some Heavy Metal Radionuclides from Soil by Bush Beans. Soil Sci. 109(4): 262-263. (with A. Wallace). 1970

Hale, V. Q. Carboxydismutase and Phosphoenolpyruvate Carboxylase Activities from Leaves of Some Plant Species from the Northern Mojave and Southern Great Basin Deserts. Ecology. (with Wallace, A., G. E. Kleinkopf, and R. C. Huffaker) (in press)

Hale, V. Q. Effect of TIBA (2, 3, 5 Triiodobenzoic Acid on Seed Production and Vegetative Growth of Medicago sativa L.) (alfalfa). Crops and Soil. (in press)

Hale, V. A. Relationships of Soil and Plants of the Nevada Test Site. Western Society of Soil Science Meeting 1970 Berkeley. Pacific Division American Association for the Advancement of Science. (with J. D. Childress) Abstract

12. SCOPE OF THE PROJECT

A soil survey - soil characterization study of some ecological study sites at the Nevada Test Site was initiated two years ago. The major objective was to assess the contribution of the soil component of the environment to the ecosystem. Soils of 76 different sites are being characterized both chemically and physically according to standard methods. Radionuclide contents of different horizons of the soil are being determined as are mineral contents of the principal types of vegetation at each site. Much of the data will be useful in developing a mineral and radionuclide cycling picture of the ecosystem represented as well as in assessing the effects of soil on the biota. The information is necessary to the overall goal of determing effects of radiation on an ecosystem.

13. RELATIONSHIP TO OTHER PROJECTS

This study is of vital importance to all the ecological projects at the Nevada Test Site including those of LRL, USPHS, and other agencies. It is also of value to the desert biome IBP studies. Soil surveys are being conducted in Nevada by the University of Nevada. A soil characterization was made in Area 18 at the Nevada Test Site by the USPHS. Our efforts have been coordinated with theirs in both cases.

14. TECHNICAL PROGRESS IN FY 1971

For the 76 sites being studied, descriptions of the profiles have been developed, the soils were assayed by standard physical and chemical procedures including for Kjeldohl nitrogen, (over 700 samples) atomic absorption analyses of chelate extracts were made of the samples, gamma ray spectrometry analyses were made for gamma - emitting radionuclides, and Coleman nitrogen and emission spectrographic analyses for minerals were made for over 700 plant samples representing the various sites.

15. EXPECTED RESULTS IN FY 1972

The data for the 76 sites will be organized into a final report of the study.

(about 300 pages) and the report as well as data on computer cards and tape will be available to all who need it. Some additional soil samples will be taken for study in Rock Valley and in Plutonium Valley and elsewhere to help characterize the soils in ^{239}Pu-contaminated areas. It was hoped that it would be possible to get soils information for more of the study sites used by Dr. Beatley.

16. EXPECTED RESULTS IN FY 1973

Termination of the projects insofar as support through GEN-12 is concerned, is planned at the end of FY 1972. It is possible that IBP and/or Nevada Operations Office may wish additional soils studies.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: CHEMICAL PROBLEMS

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

4. Date Prepared: April - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	5	5	4 ½
(b) Other Tech.	½	½	½
TOTAL:	5 ½	5 ½	5

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 58,500	$ 59,600	$ 53,600
(b) Materials and Services	21,000	14,900	14,000
Sub-Total Direct Project Support	$ 79,500	$ 74,500	$ 67,600
(c) Indirect Expenses *	41,500	41,000	34,000
TOTALS:	$ 121,000	$ 115,500	$ 101,600

* 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 1971

The Isolation of Manganese in a System Between Bis (2-Ethyl Hexyl) Hydrogen Phosphate and Aqueous Nitric Acid J. Inorg. Nucl. Chem. 32,1351 (1970)

Partition Behavior of Manganese (VII) from Aqueous Bromate Solutions of Perchloric, Formic and Acetic Acids into Bis (2-Ethyl Hexyl) Hydrogen Phosphate (HDEHP) J. Inorg. Nucl. Chem. (Submitted) 1970

12. SCOPE OF THE PROJECT

The Chemical Problems Section's primary function is the support of the program objectives of the Environmental Radiation Division and its research requirements for analytical, inorganic and radiochemistry. The second objective is to conduct continuous research in the development of new techniques, both analytical and instrumental relevant to present and future objectives.

In addition continuing programs are being carried out jointly with investigators of this Division and other Divisions. These programs have involved the preparation and radiochemical analysis of large numbers of samples during the last 8 months.

The primary effort of this section's activities during the past years has shifted from the analysis of samples by wet radiochemical methods to direct non-destructive gamma spectrometric analysis. Neutron and charged particle activation techniques have been employed on large numbers of samples for trace element content. With the exception of Sr^{90} (or other pure beta emitters) wet radiochemical analysis methods have been phased out.

13. RELATIONSHIP TO OTHER PROJECTS

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

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

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

Lamont Geological Laboratory, Columbia University

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

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

Lawrence Radiation Laboratories, Livermore, Calif.

Battelle Northwest Laboratory, Richland, Washington.

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

14. TECHNICAL PROGRESS IN FY 1971

During the first eight months of FY 1970 this Section completed the preparation of several hundred plant and water samples for radiochemical analysis in support of the Environmental Decay Section. These samples are presently being analyzed for Cs^{137}, Th, U and stable K by gamma spectrometric analysis. In addition, 650 samples were analyzed for stable Cs, Rb and Mn by neutron activation analysis in support of all Division research programs.

A number of rapid carrier-free liquid extraction methods were developed for the isolation of radio-manganese, Indium and Gallium in support of programs of this Division as well as other Divisions within the Laboratory.

15. EXPECTED RESULTS IN FY 1972

The work of this Section in a supporting role will be continued at the present level for the next fiscal year. However, all Division Research Programs involved in mixed fission product cycling or other programs requiring the analysis for gamma emitting radionuclides will be carried on jointly with the gamma spectrometry facility. The wet radiochemical analysis of these nuclides has been phased out. A 20-30% budget reduction will thus result over the next two years.

16. EXPECTED RESULTS IN FY 1973

It is anticipated that during the FY 1971 the major efforts of this Section will be in the area of stable element analysis with the expanded use of neutron, charged particles, mass spectrometry, U.V and I.R. techniques. These changes are required to give greater support to the basic research in ecology being carried out by the division investigators.

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.:	4. Date Prepared:
06-02-04	April - 1971
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA and NEVADA TEST SITE
7. Person in Charge: Arthur Wallace (Acting)	8. Project Term: From: 1971 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(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 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 33,400	$ 29,600	$ 31,600
(b) Materials and Services	9,300	6,900	8,900
Sub-Total Direct Project Support	$ 42,700	$ 36,500	$ 40,500
(c) Indirect Expenses *	24,900	24,600	25,500
TOTALS:	$ 67,600	$ 61,100	$ 66,000

* 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 1971

Koller, D. Determination of Fundamental Plant Parameters Controlling Carbon Assimilation and Transpiration by the Null-Point Compensating System (Improved Version). UCLA 12-797. 1970

Koller, D. The Partitioning of Resistances to Photosynthetic CO_2 Uptake in the Leaf. Journ. New Phytol. 69: 971-981. 1970

12. SCOPE OF THE PROJECT

Two major studies will be continued in this section. Both will be closely integrated with the desert biome studies of the International Biological Program but the studies will also include areas of interest to the AEC. The first involves fundamental aspects of the distribution of vegetation and changes brought about by various kinds of manipulation. Mathematical approaches to associations, patterning, spacing, etc. are being implemented. Shrubs and annual plants are both included. Work of this type will mostly be done in Rock Valley and Mercury Valley but consideration is to be given to other areas. A tape-recording environmental measurement station is to be established in Rock Valley. It is expected that computer interface with the tape will make it possible to develop regressions for plant (and animal) behavior with the parameters of air and soil temperature, rainfall, evaporation, wind speed and other factors. This is all part of the program for development of an ecosystem model for the area under study. The other major study concerns null-point gas exchange analysis for both CO_2 and H_2O systems for the vegetation in the field. This technique will be used as a means of testing the reliability of other methods of net productivity. Some fundamental characteristics regarding plant species found at the Nevada Test Site will be studied. Daily and seasonal cycles of photosynthesis will be measured. Changes resulting from manipulations including that of ionizing radiation will be studied. Transpiration rates will simultaneously be measured with each of the above conditions so that it will be possible to better understand water relations under conditions obtained in the field. Thermocouple psychrometer reading for soil moisture will simultaneously be made. Again these measurements are part of the total effort to develop a model for a desert ecosystem.

13. RELATIONSHIP TO OTHER PROJECTS

Gas exchange work is being done at Curlew Valley in Utah (Utah State University and Desert Biome, IBP), at Pawnee, Colorado (Grasslands IBP) and elsewhere. Glasshouse and laboratory phases of this study are being done in this laboratory (O. R. Lunt). Mathematical assessment of the distribution of vegetation is studied at many locations. Our studies are partly in relationship with David Goodall (Utah State University and Desert Biome of the IBP).

14. TECHNICAL PROGRESS IN FY 1971

A draft of a manuscript concerning the associative distribution of shrubs at the Nevada Test Site was prepared. Plots were established and considerable data taken for mathematical description of the IBP validation site in Rock Valley. Dimension analysis techniques were established for most of the major shrubs. Computer programs were developed for many phases of the project. The equipment for gas exchange studies was mounted

in a portable trailer and prepared for use in the field. The equipment was field tested and the studies started.

15. EXPECTED RESULTS IN FY 1972

The analyses of the IBP plot will be completed. The procedures will be used elsewhere on the Nevada Test Site. Several manuscripts will be developed from the data. The environmental measuring equipment will have provided a year of data and this will be assimilated with the other data towards the development of a model for the ecosystem. It is expected that the gas exchange data will lead to information for the model as well as for manuscripts.

16. EXPECTED RESULTS IN FY 1973

It is expected that all aspects of the study will be continued and refined during this fiscal year. Several years of data are needed.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: FUNCTIONAL MORPHOLOGY AND RELATIONSHIPS TO ECOLOGY

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA and NEVADA TEST SITE

7. Person in Charge: Arthur Wallace (Acting)

8. Project Term: From: 1972 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	0	0	$\frac{1}{2}$
(b) Other Tech.	0	0	$\frac{1}{4}$
TOTAL:	0	0	$\frac{3}{4}$

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 0	$ 0	$ 17,000
(b) Materials and Services	0	0	7,400
Sub-Total Direct Project Support	$ 0	$ 0	$ 24,400
(c) Indirect Expenses *	0	0	8,500
TOTALS:	$ 0	$ 0	$ 32,900

* 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 1971

None

12. SCOPE OF THE PROJECT

At the present time this is envisioned as a new project. It will concern the physiological and ecological adaptations of animals. Much of the focus will be on how these relate to ecosystem function. Relationships to radioecology are also to be developed. In part this project is in progress elsewhere and would be moved here.

13. RELATIONSHIP WITH OTHER PROJECTS

The project will be integrated with other projects in the division and also with the Department of Zoology on the UCLA campus.

14. TECHNICAL PROGRESS IN FY 1971

None

15. EXPECTED RESULTS IN FY 1972

Several aspects of the project would be developed and it is expected that reports would be developed.

16. EXPECTED RESULTS IN FY 1973

The project would be continued throughout this year. It is expected that the work would be very productive.

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.: 06-02-04

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

7. Person in Charge: George V. Alexander

8. Project Term: From: 1970 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	1	1	1
(b) Other Tech.	-	-	-
TOTAL:	1	1	1

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 15,800	$ 14,000	$ 14,000
(b) Materials and Services	5,800	2,900	3,100
Sub-Total Direct Project Support	$ 21,600	$ 16,900	$ 17,100
(c) Indirect Expenses *	8,300	8,200	8,500
TOTALS:	$ 29,900	$ 25,100	$ 25,600

* 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 1971

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 section 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 1971

The number of samples which have been analysed by our direct reading spectrometer system has been increased from 11,000 to about 19,000 during this 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 spectrometer. Several improvements have been incorporated into the method for estimating line backgrounds. A new and more stable excitation stand has been added to the system.

15. EXPECTED RESULTS IN FY 1972

The major task for FY 1972 will be to add several new elements to the analytical array. These additions will include mercury and arsenic. The sensitivity for detection of those elements in biological tissues is not sufficient to determine normal levels but is adequate to determine 'hazardous' levels.

16. EXPECTED RESULTS IN FY 1973

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 - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	2 1/2	2 1/4	2
(b) Other Tech.	-	-	-
TOTAL:	2 1/2	2 1/4	2

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 26,000	$ 25,000	$ 23,000
(b) Materials and Services	4,400	5,000	7,000
Sub-Total Direct Project Support	$ 30,400	$ 30,000	$ 30,000
(c) Indirect Expenses *	16,600	16,400	17,000
TOTALS:	$ 47,000	$ 46,400	$ 47,000

* 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 1971

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 the Medical Physics Section Activity is to develop new fundamental procedures and instruments that are specially applicable to currently important problems in radiobiologic 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 date handling and processing. Some of the new sensor developments are being used in biological and medical research applications in the Medical Physics Section under Budget Activity 06-01-01. Some emphasis will shift to cyclotron and application problems.

Some current efforts pertain to the development and application of the measurement of physical characteristics of viable cells and the use of physical method to separate cells of different characteristics.

Besides the development of a fast section scanning instrument and associated techniques planning is now under way to develop better techniques for the use of short lived cyclotron produced radioisotopes in nuclear medicine.

13. RELATIONSHIP TO OTHER PROJECTS

High Speed Section 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. Others working in various methods of section scanning are: Mr. Al Anger, Donner Lab., University of California, Berkeley; Dr. David Kuhl, University of Pennsylvania.

Physical Cell Measurement and Separation: Dr. Howard Mel, Donner Laboratory, University of California, Berkeley; Dr. Marvin Van Dilla, Los Alamos.

14. TECHNICAL PROGRESS IN FY 1971

The clinical application of fast section scanning brain, pulmonary and liver scanning has been resumed after a long delay. The anti-scalloping system is fully incorporated into the scanner.

Despite reductions in personnel and budgetary support improved instrumentation for physical cell measurements and separations have been developed which have increased the reliability, speed and resolution of the applications described under 06-01-01.

The flash holographic micrography project appears to have to be terminated due to loss of key personnel.

Close liaison with the Cyclotron Project has led to some designs of new sensing devices which can be prototyped by this Project.

15. EXPECTED RESULTS IN FY 1972

It is expected that the resources of this Project can be used to develop devices and instrumentation especially adapted to the Biomedical Cyclotron problems and the application of cyclotron produced isotopes to nuclear Medicine.

16. EXPECTED RESULTS IN FY 1973

If new improved scintillators or high atomic number solid-state detectors becomes available commercially, it is anticipated that they can be incorporated into improved nuclear medicine imaging systems, especially those for imaging, .51 MeV positron annihilation radiation. Attempts to solve this problem by other new ideas will be closely examined.

NOTE: Dr. Cassen is presently 68 years of age and may be required to retire in early FY 1972 if California legislation - generally expected to be enacted and signed into law by the governor - is, in fact, passed. As an emeritus he may still conduct an active research program. The Laboratory is expecting to support a research program under Dr. Cassen's direction in FY 1972 and FY 1973. However, the Laboratory is currently also considering an enlarged program in Radiation Instrumentation. If the enlarged program does develop, at some point we would expect the resources presently committed to Dr. Cassen to transfer to the enlarged activity. However, while Dr. Cassen is in good health the research program under his direction may be expected to be productive in both launching the cyclotron program and the enlarged program in instrumentation.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: CLINICAL NUCLEAR MEDICINE

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

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

8. Project Term: From: 1958 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	5	3 3/4	3 1/2
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	5 1/4	4	3 3/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 70,600	$ 59,000	$ 56,000
(b) Materials and Services	16,100	14,000	14,700
Sub-Total Direct Project Support	$ 86,700	$ 73,000	$ 70,700
(c) Indirect Expenses *	49,800	41,000	34,000
TOTALS:	$ 136,500	$ 114,000	$ 104,700

* 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 1971

Taplin, George V.: Radioisotope Scanning: Current Status and Some Recent Advances. Surgery Annual: 1970, 2:145-181. (Editors) Philip Cooper and Lloyd M. Nyhus. (Publishers) Appleton-Century-Crofts, Meredith Corporation, New York, 1970.

Taplin, G. V., Poe, N. D., Dore, E. K., Greenberg, A., and Isawa, T.: Radioaerosol Inhalation Scanning in Pulmonary Investigation with Radionuclides 31:296-317. (Editors) Gilson and Smoak. (Publisher) C. C. Thomas, 1970.

Benfield, John R., Isawa, Toyoharu, Nemetz, Joseph C., Johnson, DeLores E. and Taplin, George V.: Immediate, Early, and Prolonged Lung Function After Autotransplantation. Arch. Surg., 101:52-55, July 1970.

Isawa, Toyoharu, Benfield, John R., Johnson, DeLores E., and Taplin, George V.: A Comparison of Lung Scanning and Differential Bronchospirometry as Pulmonary Function Tests in Dogs, Journal of the Thoracic and Cardiovascular Surgery, St. Louis, 60:5:719-729, Nov. 1970.

Taplin, G. V.: Dynamic Studies of Liver Function with Radioisotopes, Proceedings of an International Symposium on Dynamic Studies with Radioisotopes sponsored by the International Atomic Energy Agency, Rotterdam, Holland, August 31-Sept. 4, 1970. (In press)

Isawa, Toyoharu, Benfield, John R., Castagna, John, Johnson, DeLores E. and Taplin, George V.: Functional Assessment of Canine Lung Transplants by Radioisotope Lung Scanning Procedures, American Review of Respiratory Disease, 103:76-84, 1971.

Gates, Gary F., Dore, Earl K. and Taplin, George V.: Interval Brain Scanning with Sodium Pertechnetate Tc-99m for Tumor Detectability, JAMA 215:1:85-88, Jan. 4, 1971.

Taplin, George V. and MacDonald, Norman S.: Radiochemistry of Macroaggregated Albumin and Newer Lung Scanning Agents. Seminars in Nuclear Medicine (In press) April 1971.

Poe, N. D. and Taplin, G. V.: Radioisotope Pulmonary Scanning, Nuclear Medicine. (Editor) W. H. Blahd. (Publisher) McGraw-Hill. (In press)

Isawa, Toyoharu and Taplin, George V.: Unilateral Pulmonary Artery Agenesis, Stenosis and Hypoplasia, Radiology (In press) 1971.

Isawa, Toyoharu, Hayes, Michael and Taplin, George V.: Radioaerosol Inhalation Lung Scanning: Its Role in Suspected Pulmonary Embolism, Journal of Nuclear Medicine (In press) 1971.

Isawa, Toyoharu, Benfield, John, Taplin, George V. and Johnson, Delores E.: Pulmonary Perfusion Changes After Experimental Unilateral Bronchial Occlusion and Their Clinical Implications, Radiology, (In press) 1971.

Hayes, M. and Taplin, George V.: Dynamic Kidney Function Studies in Transplant Recipients and Urological Disorders, Proceedings 2nd Nuclear Medicine Symposium, Hahnemann School of Medicine, Phila., Pa., Nov. 1970. (In press)

Hayes, M. and Maxwell, M.: The Renogram in Hypertension. Chapter in Radioisotopes in Kidney Disease. (Editor) D. Blaufox. (In press) 1971.

Hayes, M.: Preliminary Imaging Studies with a Large Crystal Scintillation Camera, Journal of Nuclear Medicine, 12:93, Feb. 1971.

Hayes, M. and Taplin, George V.: Estudios Functionales Dinamicos en Pacientes con Transplante Renal, Revista de Biologia y Medicina Nuclear (In press) 1971.

Isawa, T., Criley, J. Michael, Taplin, G. V. and Beazell, James: Early and Delayed Changes in Regional Ventilation Following Experimental Pulmonary Artery Occlusion, Journal of Nuclear Medicine, Convention issue (In press) 1971.

Isawa, T. and Taplin, George V.: Lung Imaging with Xenon-133 Gas Versus Radioaerosol Inhalation (For Aiding the Interpretation of Perfusion Scans), Journal of Nuclear Medicine, Convention Issue (In press) 1971.

Kitani, K., Taplin, G. V., Hayes, M. and Johnson, D. E.: Visualizing Biliary Excretion of Tc-99m Albumin Degradation Products (A Test of Kupffer Cell Digestive Function?) Journal Nuclear Medicine, Convention issue (In press) 1971.

Kitani, K. and Taplin, George V.: Catabolic Pathway Differences Between I-131 and Tc-99m Labeled Albumin Colloids and Microaggregates. Journal of Nuclear Medicine, Convention issue (In press) 1971.

Continued on page 119

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 of the Medical Center in the Department of Radiology has been largely accomplished with the move of the remaining personnel and equipment into the Medical Center on February 15, 1971. The Biomedical Cyclotron equipment has been received and is expected to be in operation by the 1st of April. The laboratory's clinical research efforts will be continued at Harbor General Hospital at a somewhat reduced level for the next year or two or until adequate hospital and clinical facilities 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 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 Institutes 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 Dorsey, France.

14. TECHNICAL PROGRESS IN FY 1971

Lung Studies: As noted under publications during FY 1971, review articles and/or chapters in books have been published covering the subjects Radioisotope Scanning in the 1970 Surgery Annual, Radioaerosol Inhalation Scanning in Pulmonary Investigation with Radionuclides, edited by Gilson and Smoak, published by C. C. Thomas, 1970; Dynamic Studies of Liver Function With Radioisotopes, a chapter in Proceedings of the International Symposium on Dynamic Studies with Radioisotopes, sponsored by the International Atomic Energy Agency, Rotterdam, Holland, 1970, the Biochemistry of Macroaggregated Albumin and Newer Lung Scanning Agents, appears in the April issue of Seminars in Nuclear Medicine, edited by Freeman and Blaufox, published by Henry M. Stratton, Inc., publishers, 1971.

Studies in Chronic Obstructive Lung Disease: Work in this area has been conducted largely by Dr. Isawa, now a member of the laboratory staff working at Harbor General Hospital. Three types of radioisotope lung scintigraphy have been performed along with standard tests of pulmonary function through the cooperation of Dr. Karl Wasserman of the Department of Medicine. The three lung imaging procedures have now been employed in studying thoroughly nearly 200 patients with obstructive airway disease. Several significant conclusions can be drawn regarding the role of the aerosol inhalation scan in chronic lung disease. First, this procedure appears to be more sensitive than routine lung function tests in detecting airway abnormalities. It is likely that this is so because regional, partial, and/or complete airway obstruction may exist and not be registered by routine methods because overall lung function may remain in the normal range or that the uninvolved lung may compensate for the diseased regions. Secondly, the aerosol scan patterns have two distinctly different configurations and combinations of the two which correspond with the major two types of obstructive lung disease, the emphysematous and the bronchitic, as described by Burrows and Fletcher. Thirdly, in patients with features of both types of airway disease, the bronchitic component is readily detectable in the aerosol scan pattern. The capacity to recognize this bronchitic feature or component is important because the bronchitic patient is more readily amenable to treatment than the one with pure emphysema. Another application of the aerosol scan is to study the lung clearance mechanisms by performing scans repeatedly during the first few hours following radioaerosol inhalation. Examinations of this type may well be helpful in better understanding the nature and location of abnormal airway mechanisms in chronic obstructive lung disease. Such a procedure has potential value as an indicator of the patient's response to medical treatment and may be also useful in evaluating the effectiveness of aerosolized mucolytic, antibiotic and bronchial dilator agents. Studies of this type have been performed only in a few patients to date, but the preliminary results indicate that further investigation is needed. Such studies are planned for the near future.

A RAPID SEQUENCE-PERFUSION INHALATION LUNG IMAGING PROCEDURE: With the availability of a large field size scintillation camera (Picker Dynacamera II) and two test agents with widely different energy spectra (140 KEV Tc-99m Albumin Macroaggregates and the much higher energy Indium-113m albumin solutions for aerosolization) patients with suspected pulmonary embolism are examined first for perfusion defects and if abnormalities are found the higher energy aerosol is inhaled and the patient rescanned to provide evidence for/or against underlying airway obstructive disease as a cause of the perfusion defects. The perfusion and aerosol inhalation lung imaging procedures can each be performed in all four views in less than one

hour in most patients. In patients with underlying chronic bronchitic or emphysematous disease, the diagnosis of pulmonary embolism is difficult and frequently inaccurate because chest roentgenograms are usually not helpful and lung perfusion scan findings are not characteristic of embolism. The radioaerosol scan demonstrates normal ventilation of ischemic lesions of pulmonary embolism when performed a few hours or days after embolization. On the contrary, in bullous emphysema the affected lung is neither perfused nor ventilated. Furthermore, with underlying asthmatic, bronchitic or emphysematous disease, the aerosol scan patterns are characteristic of airway versus arterial obstruction. In our experience now with over 100 patients, the combined lung perfusion-radioaerosol inhalation procedure gives adequate information when supplemented by routine clinical data and plain chest films to make an early, accurate diagnosis of pulmonary embolism in all but the most complicated cases. Finally, as a screening procedure, the radioaerosol scan serves nearly as valuable a purpose for detecting and classifying obstructive airway disease as does lung perfusion scanning in disclosing abnormalities of the pulmonary arterial circulation.

EXPERIMENTAL STUDIES IN DOGS: The functional assessment of canine lung transplants has been studied by radioisotope lung scanning procedures in 34 dogs with left lung allografts, 21 of which were fresh and 13 preserved, and in 14 dogs with autografts were studied sequentially. Both types of transplants were found to function well immediately after operation. In autografts, both ventilation and perfusion were well maintained during the period of study. In allografts, ventilation deteriorated sooner and more distinctly than perfusion. Partial bronchial obstruction at the bronchial anastomotic site was indicated on the aerosol inhalation scan as an area of excessive radioactive deposition. Such findings occurred transiently in allografts and autografts in the immediate postoperative period as a result of edema, at the time of rejection (7 to 30 days) in allografts, and many months postoperatively in autografts as a result of the bronchostenosis caused by scar formation.

Another experimental study was performed in dogs to determine the early and delayed changes in regional ventilation following pulmonary artery occlusion. It is believed that pulmonary embolism is accompanied by hypoventilation and bronchoconstriction. This concept is derived from findings obtained immediately after experimental emboliaation or pulmonary artery occlusion, but the duration of these bronchial effects is not known. Clinically, evidence of hypoventilation or bronchial constriction is rarely demonstrated by scanning. The present study was made to examine the effects of pulmonary artery occlusion on regional ventilation and to find the reason for the divergent clinical experience in pulmonary embolism with accepted physiologic concepts.

Fifteen dogs were studied in serial fashion for ventilation and perfusion changes following pulmonary artery occlusion with either a balloon catheter or with a rubber balloon filled with contrast medium. Perfusion changes were studied by using Xenon-133 gas in saline or Tc-99m or I-131 MAA. Relative ventilation was assessed either with Xenon-133 gas or with Tc-99m albumin aerosol inhaled spontaneously through an endotracheal tube. Lung imaging was performed with a scintillation camera equipped with a videotape recording system. Relative hypoventilation was found in the ischemic lung immediately and for 4-6 hours after pulmonary artery occlusion. From 6-8 hours later, ventilation returned to normal in 80 percent of the dogs and remained either normal or slightly decreased for as long as two months, unless such complications as pulmonary congestion, pneumonitis or infarction

developed. Seventy percent of the dogs studied for several weeks or more developed some type of pulmonary complication. Perfusion lung scan findings were confirmed by angiography and perfusion was shown to return when the occlusion was maintained for 48 hours or less. It was concluded that hypoventilation and bronchoconstriction occur in the ischemic lung of acute pulmonary arterial obstruction during the first few hours, but such findings do not persist unless parenchymal pulmonary complications occur.

Liver and RES Studies: Two related studies have been conducted by Dr. Kitani during the past year-and-one-half. The first has to do with Catabolic Pathway Differences Between I-131 and Tc-99m labeled Albumin Colloids and Microaggregates.

There is evidence from dog experiments that Tc-99m labeled albumin aggregates are catabolized in the liver at different rates and by different mechanisms than the same suspensions labeled with I-131. With I-131 albumin colloids (10-20 nm) externally monitored liver radioactivity levels decrease rapidly, while with the much larger size (1-5 μm) microaggregates of radioiodinated albumin the I-131 release rates from the liver are much slower. On the other hand, both the colloidal and the micron size albumin suspensions labeled with Tc-99m are removed from the liver at nearly the same rates as the I-131 microaggregates regardless of the gross difference in their mean particle sizes. (See Table).

EXTERNALLY MONITORED LIVER RADIOACTIVITY LEVELS
(Percent of Peak Activity ± 1 SD*)

	At 1.5 hrs.	At 3.0 hrs.
Tc-99m colloids	93.6 ± 3.7	87.0 ± 5.4
Tc-99m micro	95.8 ± 1.6	89.5 ± 4.9
I-131 colloids	49.2 ± 2.8	42.3 ± 3.1
I-131 micro	95.2 ± 2.6	84.9 ± 1.8

*Each group shows the average of 4 or 5 dogs

This reduction in liver turnover rates between the nm size I-131 colloids and the μm size I-131 albumin microaggregate suspensions has been attributed to gross differences in the surface area between the two particles. However, surface area differences do not explain the slow and nearly identical Tc-99m albumin colloid and albumin microaggregate liver release rates. During the period of liver release of radioactivity, blood levels show a secondary rise with the I-131 colloids and microaggregates, whereas, with the Tc-99m colloids and albumin microaggregate suspensions, only a gradual decrease in blood levels was observed.

The unique finding with the technetium labeled albumin suspensions is that their Tc-99m labeled degradation products are excreted in the bile, appearing and concentrating in the gallbladder during the first two-three hours. Furthermore, Tc-99m albumin aggregate degradation products do not accumulate in the stomach as does "free" radioiodide and pertechnetate ion. Neither do they accumulate early in the large bowel as does "free" pertechnetate. A small fraction of the dose of injected technetium appears during the first hour or two in the urinary bladder.

In conclusion, Tc-99m albumin colloidal and microaggregate suspensions are catabolized by different mechanisms within the liver than are radioiodinated albumin suspensions of the same sizes. Such suspensions give minimal radiation exposures when used for liver, spleen and bone marrow scanning. Also,

they may have a new and unique application in testing the proteolytic digestive capacity of the liver's Kupffer cells.

A second investigation--Visualizing Biliary Excretion of Tc-99m Albumin Microaggregate Degradation Products (A Test of Kupffer Cell Digestive Function?)--is now ready as a preliminary report.

This work was undertaken to examine the potential usefulness of Tc-99m albumin microaggregates for measuring the proteolytic digestive capacity of the reticuloendothelial system (RES) as opposed to radioiodinated albumin colloids and microaggregates employed previously. The intended technique was to perform repeated scan examinations of the liver, spleen and bone marrow to determine the turnover rates in each of the three major organs of the RES. Early, during the course of this study with the short-lived Tc-99m albumin preparations, biliary excretion, gallbladder concentration, and small bowel accumulation of radioactive material was observed. Therefore, the technique was changed to use the biliary excretion of the albumin microaggregate degradation products as a potential measure of the liver's Kupffer cell digestive function.

Seventy-one camera studies were performed in 47 patients with various liver and RES disorders. Twelve patients including one volunteer without known hepatic or infectious disorders served as controls. The Tc-99m albumin microaggregate suspensions (1-5 μm in diameter) were prepared from 10-50 μm albumin macroaggregates by 8-10 minutes of ultrasonic agitation. 10-15 minutes following intravenous injection of 2.5 mCi and less than 1 mg of albumin as a test dose, abdominal scanning was performed and repeated three hours later. In selected cases, serial examinations were made to observe changes in the liver and abdominal distribution of activity versus time (1-5 hours).

In the twelve controls, radioactive material gradually appears in the gallbladder and the urinary bladder within two or three hours but little, if any, enters the small intestine by this time. However, by 5 hours or later, measurable activity reaches the small intestine via the biliary tract. As additional controls, patients who were being given Tc-99m pertechnetate, albumin solutions, or albumin macroaggregates for other purposes were examined at intervals of 1-5 hours post injection for evidence of biliary excretion of the pertechnetate ion or of labeled albumin degradation products. No filling of the gallbladder or accumulation of radioactivity in the small intestine was found although small quantities did appear in the urinary bladder during the first few hours.

The biliary excretion examinations made following Tc-99m microaggregate injection showed an acceleration in the rate of excretion and an increase in the amounts of albumin degradation products entering the small intestine in patients, some with presumably overactive RES function, such as empyema, Hodgkins disease, and allergic dermatitis, whereas patients with hepatitis and cirrhosis usually had findings in the normal range or below.

In conclusion, the rate of biliary excretion of Tc-99m albumin microaggregate degradation products appears to be a clinically applicable method for studying the proteolytic digestive capacity of the liver's Kupffer cell in health and disease.

Dynamic Kidney Function Studies in Renal Transplant Recipients and Urological Disorders have been conducted at Harbor General Hospital by Dr. Hayes in collaboration with Dr. Moore of the Department of Surgery. Prior to undertaking this investigation in human renal transplant recipients a very satisfactory and useful reproducible procedure was developed for the assessment of individual kidney function by utilizing the large field of view, the dual isotope capacity, and the region of interest feature of the Picker Dynacamera II. With this instrument, it is possible to study the renal turnover of two separate agents simultaneously, radiohippuran and Tc-99m chelates which provide indices of renal blood flow and glomerular filtration, respectively. Renal turnover and bladder uptake can be measured accurately with the same equipment and test agents and applied effectively in the investigation of the kidney rejection problem in renal transplant recipients. This sequential kidney imaging and simultaneous renocystogram procedure seems to be the best way for following the course of transplant recipients and in guiding their medical or surgical management. The procedure is equally useful for following the course of patients with obstructive uropathies before and after operation. Further investigation is needed to critically evaluate the usefulness of this combined procedure in a variety of specific urological disorders.

Interval Brain Scanning With Sodium Pertechnetate Tc-99m for Improving Tumor Detectability by Gary F. Gates, Earl K. Dore and Gorge V. Taplin at Memorial Hospital of Long Beach, California. Brain scans were performed on patients during the first hour following injection of sodium pertechnetate Tc-99m, and selected patients were rescanned 1-24 hours later. Eighty brain tumors were found among nearly 1,500 patients scanned during a two-year period. Diagnoses were established for 74 through histological examination, and 6 through clinical, scan, and neuroradiological findings. Forty were scanned twice, and in 15 the early scans were normal or equivocal. Fourteen of these 15 had abnormal findings upon rescanning. Twenty-six nondiagnostic to moderately abnormal early scans increased in their degree of abnormality upon rescanning. The detectability of brain tumors improved from 80 percent with routine early scanning, to 93 percent with rescanning. The optimal time for repeated scanning is 3-4 hours following tracer injection.

15. EXPECTED RESULTS IN FY 1972

Lung Function Studies: Lung function studies in chronic obstructive airway disease, bronchogenic carcinoma, and pulmonary embolism will be continued by Dr. Morinari who will be replacing Dr. Isawa in July 1971. Greater emphasis will be placed on sequential radioaerosol inhalation scanning in regard to gaining further 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 having 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, a study of chronic obstructive pulmonary diseased patients will be studied with the objective of determining reversible versus irreversible localized disease. Separate from the clinical investigations in patients, normal human volunteer subjects will be studied with aerosols of different particle size distribution to determine differences in bronchopulmonary aerosol deposition patterns in relation to the size of the aerosol inhaled. Concurrently, animal studies (dogs) will be made for the same

purpose using aerosols of killed bacteria and spores of increasing size range from 0.5 to 10.0 μm. A corollary of these studies will be an attempt to develop a radioisotope method of bronchography along with means to measure rates of lung clearance from various regions of the respiratory tract.

Liver and RES Studies: Work in this area will be continued by Dr. Kitani during the Summer and early Fall of 1971 and later by Dr. Hayes and the NIH supported nuclear medicine fellow. Further investigation of the digestive capacity of the RES cells in the liver, spleen, and bone marrow will be made using radioiodinated microaggregated albumin using Iodine-123 when it becomes available from our Biomedical Cyclotron Facility. Further clinical investigations of the use of sequential upper abdominal scanning with radioiodinated rose bengal will be continued to obtain further information regarding the clinical usefulness of this procedure in distinguishing medical from surgical jaundice.

Kidney Function Studies: Work in this area will be continued by Dr. H. Hayes, consultant to the laboratory, the person in charge of scanning studies at the Harbor General Hospital. Dr. Hayes plans to continue his collaborative studies with Dr. Moore in investigating the rejection phenomenon in renal transplantation in dogs as well as in patients. In these studies he will be using the same equipment and techniques as described for his work during 1971.

Quantitative Measurement of Right to Left Heart Shunting: Work began on this procedure in the latter part of FY 1971 by Drs. E. K. Dore, G. F. Gates, and G. V. Taplin at Memorial Hospital at Long Beach. Work will be continued to further accumulate data on the method of quantitatively measuring right to left heart shunting by whole body camera scintigraphy following the intravenous injection of specially prepared Tc-99m albumin macroaggregates of a size and fragility which make them almost completely innocuous (estimated safety factor greater than 20,000) as a test agent for lung and whole body imaging. The huge safety factor in respect to potential cerebral complications is calculated from thorough histological studies of brain sections in monkeys reported in 1966 wherein the safety factor was estimated to be greater than 2000 using I-131 MAA aggregates in the 10-60 μm range. The currently used technetium labeled MAA particles are prepared to the 10-40 μm range and are known to be far more susceptible to ultrasonic agitation than are the larger and harder appearing iodinated aggregates. The amount of whole body radioactivity versus that recorded in the lungs is an accurately measurable ratio which gives the fraction of blood delivered to the right heart which is instantly shunted to the general circulation. Results of this procedure are to be correlated with standard methods involving measurements made at heart catheterization and by other isotopic methods previously described by Wagner and other groups.

16. EXPECTED RESULTS IN FY 1973:

Lung Studies: Most of the lung function studies conducted during FY 1972 are likely to be continued by Dr. Morinari again in collaboration with Dr. Karl Wasserman of the Pulmonary Function Laboratory and with Dr. Daniel Torrance, Chief of Radiology at Harbor General Hospital. Emphasis will be continued on the development of better methods for evaluating abnormalities of lung clearance mechanisms and for the development of new procedures and materials for devising a useful method of radionuclide bronchography.

Studies on the RE System, kidney function assessment in transplanted recipients and urological disorders are planned to be continued although it is possible that by this time most of the research activity currently conducted at Harbor General Hospital may be transferred to the Medical Center. In this event, it is anticipated that a considerable effort will be under way to utilize and evaluate short-lived nuclides and organic compounds as they become available from our cyclotron facility and radiopharmaceutical development 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: HEMODYNAMICS

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

4. Date Prepared: April - 1971

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: 1971

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2	0	0
(b) Other Tech.	1/4	0	0
TOTAL:	2 1/4	0	0

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 34,100	$ 0	$ 0
(b) Materials and Services	7,400	0	0
Sub-Total Direct Project Support	$ 41,500	$ 0	$ 0
(c) Indirect Expenses *	16,600	0	0
TOTALS:	$ 58,100	$ 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 1971

Kennady, John C., Miller, B. J., Anderson, W. B., and Hayes, M.: Comparative Assessment of Cerebrovascular Disease by Angiography, Scanning, and Image Intensifier Camera, Journal Nuclear Medicine 11:333, 1970

Kennady, J. C., Anderson, W. B., and Hayes, M.: Brain Hemisphere Hemodynamics by Image Intensifier Videocamera, Society of Nuclear Medicine, 17th Annual meeting, Washington, D. C., July, 1970. "Honorable Mention Award." (Scientific Exhibit)

Kennady, J. C.: Hemisphere Assessment With the Image Intensifier Videocamera; Proceedings of the International Symposium on Cerebral Blood Flow, Royal College of Physicians, London, England, Sept. 17-19, 1970. Pitmans Medical Publishers, London, 1971.

Kennady, John C.: Advantages of Kinetic Compared with Static Imaging of Intracranial Lesions. Journal of Nuclear Medicine (In press), 1971.

Kennady, John C.: Rapid Assessment of Cerebral Hemodynamics. In J. P. Shade (Ed.), Cerebral Blood Flow, Progress in Brain Research; (Publishers) Elsevier, Amsterdam, 1971.

12. SCOPE OF THE PROJECT

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

The research activities of this section during this past year have been advancements in the quantitative assessment of cerebral blood flow. This has been accomplished by a small bolus internal carotid injection of Tc-99m pertechnetate and iconoscopic recording of the test agent passage through the cerebral hemisphere. The videoquantifier is used for regional quantification of blood flow and three results are 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 goals 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. RELATIONSHIP TO OTHER PROJECTS

Studies in measurement of cerebral blood flow are being conducted at Loma Linda School of Medicine by Dr. George Austin, at the University of Pennsylvania by Dr. Langfitt; at Baylor University by Dr. John S. Meyer; in England by Drs. Harper, Jennett and Symon; in Hannover, Germany by Dr. M. Brock; in Sweden and Denmark by Drs. 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 Dr. Wilson; at the University of Kentucky by Dr. Norrell; at Duke University by Dr. Mahaley; in Canada by Drs. Morley and Tator.

14. TECHNICAL PROGRESS IN FY 1971

Iconoscopic Studies: During this period, 27 patients with no EEG or angiographic abnormalities and no abnormal neurological findings implicating the hemisphere studied have formed the control group. The percent mean of the maximum Tc-99m concentration was determined for 29 regions (1.5 cm^2) in each patient. The mean of these percent mean maximum concentrations and one standard deviation was then ascertained. Also the mean inverse T-1/2 was derived for all the inverse T-1/2 values for that area and one standard deviation. These values indicate that the highest blood flow rates normally are in the superior and posterior parietal regions and the lowest are in the inferior frontal and temporal areas.

Patients with various types of intracranial pathology (i.e., acute and chronic vascular disease, vascular and avascular tumors, cystic tumors, arteriovenous malformations, idiopathic seizures and brain trauma) have been studied. These regional blood flow values have been compared with corresponding area values in the control group and considered abnormal if greater or less than three standard deviations apart. The results have been highly accurate in assessing the location and extent of the hemisphere lesion in these patients. Moreover, the type of pathology can be predicted from these results with a high degree of fidelity prior to operation.

All of these patients have had carotid angiography with selective catheterization of the external and internal carotid arteries. The correlation of angiographic findings with the camera results is excellent. There have been instances in which the major cerebral artery was displaced and a problem of discriminating between a primary versus secondary brain tumor. The iconoscopic studies have consistantly shown the secondary tumors to be avascular regions surrounded by a zone of hyperemia and the low grade primary tumor to have some degree of vascularity with no surrounding region of increased blood flow. In two of these patients the routine brain scan was reported within normal limits.

Human Brain Tumor Studies: Further work has been done using autoradiographic techniques on human brain tumor cells cultivated on glass cover slips. Cells that initially were interpreted as belonging to the connective tissue stroma of the tumor have been found to be, in many instances, variants of the tumor cell. At the present time, cell cloning is being carried out as well as cell phase control in order to ascertain the optimal time for specific cell affinity for the radiopharmaceutical.

15. EXPECTED RESULTS IN FY 1972

This project will be terminated but may be reactivated at a later date when possible.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: BASIC NUCLEAR MEDICINE

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

4. Date Prepared: April - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	3	3	3
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3 1/4	3 1/4	3 1/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 35,400	$ 40,000	$ 41,000
(b) Materials and Services	11,100	12,500	13,600
Sub-Total Direct Project Support	$ 46,500	$ 52,500	$ 54,600
(c) Indirect Expenses *	24,900	32,800	25,500
TOTALS:	$ 71,400	$ 85,300	$ 80,100

* 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 1971

Taplin, G. V., N. D. Poe, E. K. Dore, A. Greenberg, and T. Isawa: Radioaerosol Inhalation Scanning, Pulmonary Investigation with Radionuclides, 31:296-317; editors, Gilson and Smoak. Publisher, C. C. Thomas, 1970.

Poe, N. D.: The Influence of Pulmonary Arterial Ischemia on the Distribution of Inhaled Radioaerosols. J. Nucl. Med. 11:351, 1970.

Poe, N. D.: Cardiodynamic Effects of Intracoronary Artery Injection of Albumin Macroaggregates. J. Nucl. Med. 11:6:350, 1970.

Poe, N. D.: Influence of Coronary Arterial Injection of Radioactive Particles on Cardiodynamics. Clinical Research 19:1:118, Jan. 1971.

Poe, N. D. and G. V. Taplin: Radioisotope Pulmonary Scanning, Nuclear Medicine; editor, W. H. Blahd, McGraw-Hill. (In press)

Upham, T., S. Graham, R. Steckel, and N. Poe: Determination of In Vivo Persistence of Tantalum Dust Following Bronchgraphy. Am. J. Roentgenology. (In press)

12. SCOPE OF THE PROJECT

The primary goal of this section is to study basic physiologic and pathologic problems with radioactive materials with the intent of applying the resultant information to clinical diagnostic radioisotope tests. The feasibility, safety, and efficacy of new procedures are also examined. In this regard, this section functions as an experimental testing facility for other sections in the Nuclear Medicine Division. In addition and on a limited scale, the facilities of this section are made available to investigators elsewhere in the UCLA Medical Center who could benefit from radioisotope examinations in their research. In return, we gain professional, technical and material support from these individuals.

In July 1971 a major conjoint scientific effort with the Division of Cardiology is envisioned, with the purpose of developing an improved scanning technique for detecting injured, infarcted, or scarred myocardium. This project will center around short-lived, cyclotron-produced materials which will concentrate in either normal or abnormal cardiac tissue. The magnitude of this project will depend on the extent of outside support generated by Cardiology and the availability of appropriate radionuclides. Studies in this section are already directed toward this goal.

Continued interest remains in the potential use of radioaerosol scanning in the evaluation of broncho-pulmonary disease. More effort will be made in animal models to define the mechanism of increased aerosol deposition at sites of partial bronchial obstruction and to determine the value, if any, of serial inhalation scans during therapy of obstructive airway disease in humans. Because there continues to be no satisfactory method for measuring the particle size of wet aerosols, comparative distribution patterns of retained intrapulmonary radioaerosols from various aerosol generators will be analyzed in an attempt to develop an alternative method of characterizing these particles.

13. RELATIONSHIP TO OTHER PROJECTS

The myocardial infarction study is related to ongoing work by Ashburn at the University of California, San Diego; Wagner at Johns Hopkins; and Winchell at Berkeley. Radioaerosol scanning research is being carried out by Newhouse in Montreal, Pircher in Houston, and Taplin and Isawa in Los Angeles.

14. TECHNICAL PROGRESS IN FY 1971

(a) Effect of Glucose-Insulin Infusion on the Distribution of Cesium-131 in Experimental Myocardial Infarction. The integrity of the cell membrane in damaged myocardium is reduced leading, among other things, to the loss of intracellular K^+. A glucose-insulin-potassium solution given intravenously has been suggested as a treatment for acute myocardial infarction by returning K^+ to the damaged cell, possibly thereby improving its function. The clinical results have been equivocal. However, the theory behind K^+ restoration is logical. This principle was employed in an attempt to produce a "hot spot" on the scan around an area of infarction in dogs. Following a baseline scan with the K^+ substitute ^{131}Cs to outline the infarcted area, a glucose-insulin infusion was begun. The results were opposite those expected. The cold infarcted area tended to increase in size with signs of increased local ^{131}Cs deposition in the normal tissue. However, the experiment did demonstrate that relatively acute subtle changes in ionic distribution could be detected by scanning even in the beating heart.

(b) Regional Coronary Flow Distribution Outlined by Intracoronary Injection of Cesium-131. Previous work in this section has shown that carefully prepared radioalbumin macroaggregates in small amounts can be directly injected into the coronary arteries of dogs with no detectable alteration in cardiodynamics. This technique has subsequently been used elsewhere in humans. However, transient blockade of coronary vessels is accompanied by obvious hazards. To circumvent these, comparative observations between the distribution of ^{99m}Tc-albumin particles and ^{131}Cs solutions have been made. Preliminary observations show good correlation. If confirmed, the use of ionic solutions is to be much preferred.

(c) Radioaerosol Deposition in Experimental Vascular Obstruction. Earlier studies were expanded in an attempt to determine if true differences result in radioaerosol deposition in ischemic lungs (experimental pulmonary embolization with Ivalon sponge) when aerosol is administered by positive or negative pressure in anesthetized animals. Although the results are borderline, it appears that better deposition is achieved with positive pressure.

15. EXPECTED RESULTS IN FY 1972

(a) Myocardial Infarct Study. Scan studies of myocardial infarction in intact animals and in humans will be performed using cyclotron-produced ^{129}Cs or ^{43}K. The distribution patterns will be compared to near simultaneous coronary angiography in patients. This work will be carried out with the Cardiology Division. If the results appear promising, investigations with ^{11}C labeled fatty acids or carboxylates will be carried out with the purpose of developing a means of frequent re-evaluation of regional coronary flow and myocardial function in infarct patients. With the short-lived ^{11}C compounds, studies could be repeated almost hourly.

(b) Comparison of Regional Coronary Blood Flow Techniques. Studies begun last year will be continued to determine the relationship between the

distribution of ^{99m}Tc albumin macroaggregates and ^{131}Cs solutions after coronary arterial injection. The particle method theoretically is superior because it actually measures flow through any capillary bed. ^{131}Cs and other K^+-like materials depend on intact cells to take up and hold the ions. Damaged cells may not concentrate ^{131}Cs even if the blood flow is relatively good.

(c) Exploration of "Hot Spots" at Sites of Partial Bronchial Obstruction after Inhalation of Radioaerosols. Increased aerosol deposition at sites of partial bronchial obstruction may be due to increased particle impaction from turbulence or to pooling of secretions from defective ciliary activity or to a combination. Each of these factors will be explored in animal models.

(d) Value of Delayed Scans after Radioaerosol Administration. Exploration of this area, which was discontinued several years ago due to lack of a suitable semi-short-lived isotope, will proceed with availability of ^{123}I or ^{111}In. Both of these elements, as less desirable radionuclides, have previously been used for aerosol studies. The major advantage of the delayed scan is thought to be in demonstration of alveolar deposition which in turn is an indicator of ventilation.

16. EXPECTED RESULTS IN FY 1973

The direction of activities at this time will be reflected by the success obtained during the previous year. Major emphasis will be placed on noninvasive techniques for evaluating myocardial infarction and coronary blood flow. It is most likely that ^{11}C compounds will receive major attention, but agents containing isotopes of better energy and longer shelf life, for example, ^{123}I fatty acids, will be investigated because of their potentially more widely available utilization. A similar approach can be applied to cancer localization. The probability of achieving tumor uptake of a labeled compound seems more likely than the probability of uptake by injured or infarcted myocardium.

Radioaerosol inhalation and perfusion lung scan studies will continue to determine the place of these examinations in evaluation of therapy in chronic pulmonary diseases.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: NUCLIDE METABOLISM

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

7. Person in Charge: Norman S. MacDonald

8. Project Term: From: 1955 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2 3/4	2 1/4	2 1/4
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3	2 1/2	2 1/2

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 35,200	$ 32,000	$ 32,000
(b) Materials and Services	6,700	8,200	8,200
Sub-Total Direct Project Support	$ 41,900	$ 40,200	$ 40,200
(c) Indirect Expenses *	24,900	24,600	25,500
TOTALS:	$ 66,800	$ 64,800	$ 65,700

* 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 1971

Levy, J., Barnett, E. V., MacDonald, N. S., and Klinenberg, J. R.: Altered Immunoglobulin Metabolism in Systemic Lupus Erythematosus and Rheumatoid Arthritis. Journal Clinical Invest. 49:708-715, 1970.

MacDonald, N. S., Ban, I., Flesher, A. and Hackendorf, M.: Cesium-137 Levels in Human Adult Population in California, 1960-1969. Nature, 228:283-284, 1970.

MacDonald, N. S., Hackendorf, M. and Flesher, A.: Placental Transfer of Tin-113 Contamination in Indium-113m Generator Eluates. Journal of Nuclear Medicine 11:344, June 1970.

Taplin, George V. and MacDonald, Norman S.: Radiochemistry of Macroaggregated Albumin and Newer Lung Scanning Agents. Seminars in Nuclear Medicine (In press) 1971.

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 1971

The rates of turnover of immune globulin G and M were determined in 49 patients with rheumatic disorders. These studies performed in collaboration

with Dr. Joshua Levy and others of the Department of Medicine, are aimed at elucidating the causes of rheumatic diseases and providing methods for objective evaluation of the efficacy of their treatment. Our contribution to the program consists of measuring the turnover rates of the two proteins (which have been tagged with radioactive Iodine-131 and Iodine-125, respectively), in blood and in the total body over a period of 3 weeks following their intravenous administration. These radioassay data from serial blood samples and total body counting, are then combined with other information obtained by the other collaborators (such as serum concentrations of the two immune globulins, plasma volume and body weight) to permit calculation of the daily rates of synthesis and of catabolism of these proteins. By performing these determinations before and after chemotherapy, it was found, for example, that Azathioprene suppressed the daily synthetic rate of IgG by as much as 74 percent in 7 of 9 patients. Production of IgM was reduced by like amounts in 8 of 9 patients. The concentrations of these proteins in the plasma were not greatly affected, probably because their rates of degradation also decreased during the 4-6 month treatment, thus compensating for the drop in synthesis. These changes were accompanied by clinical improvement in the patient's condition.

The Total Body Counter (TBC) was also employed to determine potassium levels in the body by measuring the natural K-40 radionuclide. For example, the amount of natural potassium was determined in the bodies of 6 young male volunteers before and after their confinement for three months in a full-scale model of an experimental life-support system module designed for space exploration. The potassium measurements were but a small part of the extensive program conducted by the McDonnell-Douglas Corporation. Our significant contribution was the finding that each of the three men who did not exercise actively during their confinement lost about 10 grams of potassium.

Programmed physical exercise by the others resulted in maintenance of their normal body potassium level. In another collaborative effort with Drs. Maloney and Henney of the Division of Thoracic Surgery of the School of Medicine, repeated measurements of body potassium were made on 40 dogs during the course of various experimental procedures. It was found, for example, that dogs can be rapidly depleted of 20-25 percent of their total body potassium stores by means of special diets. Our capability for measuring changes of as little as two grams in fifty of natural potassium with a standard deviation of $\pm$ 10 percent proved valuable to the research program of this group. A number of procedures of diagnostic value were performed with the Total Body Counter facility as a service to the clinical staff of the Hospital. These included determinations of body potassium depletion, if any, in patients with subnormal plasma K concentrations; measurements of serum albumin turnover rates, and of iodide uptake in infants using I-131 as tracer; and measurements of iron absorption and turnover with Fe-59. Rates of clearance of tantalum particles (tagged with Ta-182), from the lungs of dogs following their administration for radiographic purposes were also conveniently determined with the TBC.

The monitoring of Southern California adult residents since the last report (Nature, Oct. 17, 1970) revealed that the mean quarterly values for Cesium-137 contamination in the bodies of males during 1970 was 26 pCi per gm of K, and 23 pCi/gm K for females, (total measurements - 186.) Thus, the rapid downward trend from the peak in 1965, which flattened out during 1968-1969, has indeed been replaced by a flat trend during 1970. The levels of Cs-137 in these people are not decreasing, and there is some evidence that their body burdens may even be increasing very slightly. It must be emphasized

that the accrued radiation dose to a person from these very small amounts of Cs-137 over the last decade was calculated to be less than 6 percent of the absorbed radiation dose delivered by the radioactive, natural, K-40, inescapably present in his body.

15. EXPECTED RESULTS IN FY 1972

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. These will include Cs-129, In-111, Ga-67, K-43, C-11 and other cyclotron-produced radionuclides.

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.

16. EXPECTED RESULTS IN FY 1973

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 "new" short-lived radionuclides by the Biomedical Cyclotron Facility is anticipated. 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 - 1971
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: George V. Taplin, M.D. and Norman S. MacDonald	8. Project Term: From: 1967 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	2 1/2	3 3/4	3 3/4
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	2 3/4	4	4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 42,300	$ 56,000	$ 56,000
(b) Materials and Services	9,100	12,100	12,100
Sub-Total Direct Project Support	$ 51,400	$ 68,100	$ 68,100
(c) Indirect Expenses *	33,200	41,000	34,000
TOTALS:	$ 84,600	$ 109,100	$ 102,100

* 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 1971

None

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 1971

One of the first nuclides to be produced with the Biomedical Cyclotron will be Gallium-67 for clinical studies in tumor visualization by gamma scintigraphy. One approach to be attempted will be through the bombardment of copper foil targets (Cu-65) with helium $^{-3}$ ions. A chemical method for separating the desired product from the target material was developed. This involved dissolving the target after bombardment in hydrochloric acid, and extraction of the Gallium with a n-heptane solution of bis-2-ethyl hexyl hydrogen phosphate. Stripping of the organic layer with HCl yielded a solution of Ga completely free of copper. The method is rapid and adaptable to remote handling techniques. The same solvent system was found also to be effective in separation of Indium from silver target foils. This will be utilized if our future attempts to prepare Indium-111 (also for tumor localization scanning) by alpha particle, or He-3, bombardments of silver prove successful. A titanium target chamber to prepare Fluorine-18 (as an aqueous solution of fluroide ion) by He-3 bombardment of water was procured from the Cyclotron Corporation (based on a design by Sloan-Kettering Institute). Several modifications were then made so that the product solution can be removed and transferred to the hot labs via teflon tubing by remote control; and a system was devised to process and dispense this solution into individual portions in sterile, pyrogen-free form suitable for use in humans.

Indium-113m is becoming widely used in nuclear medicine as a short-lived radioactive label in a number of scanning procedures. The accepted view regarding the transport of ionic indium in the blood following intravenous injection is that it is bound to transferrin and transported like ferric iron. In collaboration with Dr. T. G. Hennessy, a critical study of this theory was initiated. Rabbits received intravenous injections of a mixture of Iron-59 and either Indium-113, or Indium-114 (when more prolonged time intervals were desired) together with sufficient rabbit serum to buffer the

acid solutions. Preliminary results of serial blood sampling showed that Indium disappeared from the blood at rates describable by the sum of two exponential functions with half-times of 10-15 minutes and 7-8 hours whereas Fe-59 always cleared at a single exponential rate with a half time between 1-2 hours. Other differences appeared in the distribution of the two nuclides in the liver, spleen, bone marrow, and erythrocytes. It may be possible to account for these differences on the basis that the iron is utilized in erythropoesis whereas the indium is not.

15. EXPECTED RESULTS IN FY 1972

Special efforts will be devoted to establishing procedures for processing the radionuclides made with the cyclotron into forms acceptable for human usage. For example, gas chromatographic techniques will be adapted to establish the chemical identity and purity of all gaseous products intended for administration to humans (radioactive CO_2, N_2, oxygen-15, etc.) as well as the radioisotopic composition. All solutions likewise will be subject to a control program set up to insure conformity to radiopharmaceutical standards.

Labeling of various types of organic compounds with Carbon-11 will be undertaken following, at first, certain established procedures such as the Grignard synthesis of simple carboxylic acids. Efforts will also be directed to the labeling of certain essential amino acids with Carbon-11 and possibly Fluorine-18. The goal of this work is to obtain specific compounds of physiologic significance, labeled with short-lived gamma-emitting nuclides for kinetic studies and for organ visualization in patients.

16. EXPECTED RESULTS IN FY 1973

The major activity will continue to be the development of means of converting "raw" radionuclides into radiopharmaceutical agents suitable for administration to humans for the clinical research programs in nuclear medicine. Adequate supplies of Iodine-123 will, hopefully, have become available. This radionuclide will receive special attention because it can be used to advantage immediately in currently active clinical programs, both in its ionic form, iodide, and in a variety of well-known, labeled compounds such as Rose Bengal, Hippuran, and serum albumin and globulins.

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 - 1971

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 1971	FY 1972	FY 1973
(a) Scientific	2	2	2
(b) Other Tech.	1/4	1/2	1/2
TOTAL:	2 1/4	2 1/2	2 1/2

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 23,000	$ 26,000	$ 27,000
(b) Materials and Services	8,700	13,100	14,200
Sub-Total Direct Project Support	$ 31,700	$ 39,100	$ 41,200
(c) Indirect Expenses *	16,600	16,400	17,000
TOTALS:	$ 48,300	$ 55,500	$ 58,200

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

11. PUBLICATIONS DURING FY 1971

Douglas, Clayton H., Edgar L. Surprenant, Mary A. Grossnick, and Milo M. Webber: Three-year Experience with 99m-Tc-Albumin Preparations in a Community Hospital. J. Nucl. Med. 11:6:387, 1970 (abstract).

Cragin, Michael D., Milo M. Webber, Winona K. Victery and Daniel Pintauro: Technique for the Rapid Preparation of Lung Scan Particles Using 99m-Tc-Sulfur and Human Serum Albumin. J. Nucl. Med. 11:6:385, 1970 (abstract).

Webber, Milo M. and Joseph Wagner: Demonstration of Vascularity of the Femoral Head Using Technetium-Sulfur Colloid. J. Nucl. Med. 11:6:376, 1970 (abstract).

Webber, Milo M., Michael D. Cragin, Julius H. Grollman, Roscoe C. Webb, and Leslie R. Bennett: Demonstration of Thrombophlebitis Using Radioisotope Techniques. J. Nucl. Med. 11:6:375-376, 1970 (abstract).

Wagner, M. S., L. R. Bennett, G. Kantor, and T. Stanley: Scintillation Camera Evaluation of Renal Function Changes after Percutaneous Renal Biopsy in Dogs Using 99m-Tc-DTPA and 131-I-Hippuran. J. Nucl. Med. 11: 6:372, 1970 (abstract).

Wagner, M. S., L. R. Bennett, H. Kramer, and H. Gonick: 99m-Tc-DTPA: A Comparison with 131-I-Hippuran for Gamma Camera Renal Studies in Chronic Renal Disease. Radiology (in press).

12. SCOPE OF THE PROJECT

This project is designed to study the clinical applications of short-lived radionuclides. The first such nuclide introduced into general medical use, Technetium-99m, revolutionized the field of radioisotope scintigraphy. Development and refinement of clinical methods utilizing such short-lived isotopes are the goal of this project. Work has included the evaluation of Technetium-Sulfur suspension incorporated into macroaggregates of albumin as a lung scanning agent; the long-term evaluation of Fluorine-18 in bone scanning; and the study of Indium-111 in the demonstration of soft tissue disease such as that caused by neoplastic processes. To obtain standards for clinical diagnosis, the appearance of "normal" lung scans is being studied.

A major concern of this project is the detection of intravascular fibrin deposits by the use of labeled fibrinogen and comparison of this study with those previously developed, including the localization of macroaggregates of albumin on thrombosis sites and radiographic venography. Another subject of investigation is the use of antibodies for studying sites of fungal infection in various organs.

The operation of the cyclotron will shortly make available radioactive materials with very short half-lives. The preparation of Carbon-11 labeled fatty acids, which are quickly metabolized by the heart, may allow detection of cardiac muscle disease. Preparation of Carbon-11 labeled amino acids for uptake by the pancreas may provide an improved means of evaluating the morphology and function of that organ. New radioactive gases may become more practical in the evaluation of various portions of the lung than has been the longer-lived radioactive Xenon gas.

13. RELATIONSHIP TO OTHER PROJECTS

This project is related to the radiodiagnostic agent development sections and to those of the basic nuclear medicine sections. Short-lived Technetium compounds, especially colloids of Technetium sulfur, are being used in many clinical laboratories throughout the United States. Refinements and development of other uses for such colloids are related to projects in other laboratories. The labeled fibrinogen project mentioned is related to a USPHS grant project entitled "Radioscintigraphy Demonstration of Thrombophlebitis", number 5 RO1-GM-17113, and is considered to be complementary to it. Related to this project is the evaluation of the normal variations of the lung scan.

14. TECHNICAL PROGRESS IN FY 1971

The effect of aluminum concentration on stabilized Technetium-Sulfur colloid compounds has been explored. Many different Technetium/Molybdenum generators are used in various laboratories throughout the country. The amount of aluminum to be expected in the eluates of these generators was evaluated and tabulated. Comments in the literature suggest that the aluminum content is detrimental to the formation of particles of Technetium-sulfur colloid and have suggested the routine adding of chelating agents. Our evaluation of the quality of the scans obtained suggests that the albumin stabilization of particles prepared by our previously reported method is not influenced by the presence of aluminum at the levels usually encountered.

A Technetium lung scanning agent which has been used in this laboratory, consisting of particles of albumin incorporating smaller particles of radiotechnetium sulfur suspension, has been evaluated as to any possible deleterious effect on patients who have received it. The charts of 100 patients were reviewed in great detail with careful consideration of any evidence of adverse effect that could have been attributed to the lung scanning agent. No evidence of such effects was apparent from the study.

A series of approximately 45 patients has been evaluated to determine the normal appearance of the lung scan as done with macroaggregates of albumin. The patients were not suffering from any known pulmonary or cardiac disease. They were examined in the usual routine views and the results tabulated. It was found that approximately five percent of the "normal" patients showed perfusion defects great enough to be considered suggestive of a disease process. The significance of these findings is not altoghether clear. It may mean that there is a variation of the normal due to anatomic differences in blood supply to the lungs, or it may mean that a certain degree of pulmonary embolization is normal. There are, of course, other explanations, and in a continuation of this phase of the project we expect to evaluate these patients to determine whether there is any disease detectable by other methods.

Antibodies against coccidioidomycosis have been prepared, labeled, and administered to mice which had been previously infected with coccidioidomycosis in focal areas. Distribution of the labeled substance was then assayed by *in vitro* methods to determine whether significant localization of the tracer might have been achieved as a result of the antigen/antibody

reaction. The results so far appear to be disappointing in that little if any selective localization of tracer is apparent over and above the localization of labeled non-specific antibody. Work is continuing in the use of such antibodies as carriers of tracers to points of interest.

The use of radiolabeled tracers to demonstrate whether renal biopsy significantly affects the function of the kidney was studied. Renogram curves were obtained before and after renal biopsy; careful correlation suggests that a temporary effect on renal blood flow occurs in some cases that have undergone biopsy.

The use of Gallium-67 has been explored in patients having tumors involving soft tissue. Several very clear demonstrations of lymphomas have been achieved using this substance. At the present time an insufficient number of patients have been accumulated to determine whether this technique has clinical prognostic value. Similarly, the use of Indium-111 for tumor localization has recently been instituted. In addition, Indium-111 has been used in the colloidal form for lymph node localization and as a scanning agent for recording the flow of cerebral-spinal fluid. The latter studies have been performed in animals in order to determine whether local areas of meningitis might be demonstrated by an accumulation of the particles at the site of inflammation. The result of this work is still pending.

There has been a great deal of activity in the use of radiotracers injected into the blood stream via a peripheral vein and the following of the course of these tracers through the heart and lungs in order to demonstrate the presence or absence of abnormal shunts between the various chambers of the heart. Work along these lines suggests that there is much promise in these techniques. It may be that they will serve as a screening procedure prior to cardiac catheterization.

Also underway is an evaluation of area-of-interest studies and the scintillation camera to diagnose cardiac flow impairment. This technique is based on the difference in the transit time through the heart as compared to a large vessel such as the aorta just beyond the heart. The holdup of tracer in the heart leading to an increase in transit time is influenced by the amount of recirculation in the heart through the coronary arteries. The method shows promise of estimating whether coronary blood flow falls into the normal range.

15. EXPECTED RESULTS IN FY 1972

In conjunction with the surgical service, we will continue work on the correlation of blood clot localization studies using tagged fibrinogen with scanning methods, as well as with radiographic venography. Pre- and post-operative pulmonary scans on patients who have undergone surgery will be studied to determine the significance of perfusion deficits shown on the lung scan. Dynamic flow studies will be applied to cardiac problems to determine the extent of their value in the diagnosis of congenital and acquired cardiac disease. It is hoped that availability of short-lived tracers from the cyclotron will make possible the tagging of fatty acids which can be used to demonstrate areas of metabolic activity as correlated with blood flow, as in the myocardium. Studies of vascularity of the femoral head in patients with known hip disease have been deferred until this year. Technetium-sulfur colloid has proved to be a good index

of vascularity of the femoral head in normal individuals; in cooperation with the Department of Orthopedics we expect to apply all available radionuclide agents to the difficult problem of predicting the vascularity and viability of the femoral head following fractures.

16. EXPECTED RESULTS IN FY 1973

Adequate cyclotron operation at this time is expected. Carbon-11, Oxygen-15 and Nitrogen-13 will be used in dynamic studies such as determination of their pulmonary distribution. Carbon-11/amino acid compounds, which need to be synthesized quickly because of the short tracer half-life, may prove valuable in demonstrating areas of protein synthesis of the body, such as the pancreas, parathyroid glands, and some tumors. Colloidal particles containing high levels of short-lived tracers may demonstrate the bone marrow much better than is now possible.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: BIOMEDICAL CYCLOTRON FACILITY

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

4. Date Prepared: April - 1971

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

6. Working Location: UCLA

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

8. Project Term: From: 1969 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	4	5	5 1/2
(b) Other Tech.	1/4	1/4	1/2
TOTAL:	4 1/4	5 1/4	6

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 61,500	$ 74,000	$ 83,000
(b) Materials and Services	17,000	28,500	40,700
Sub-Total Direct Project Support	$ 78,500	$ 102,500	$ 123,700
(c) Indirect Expenses *	49,800	49,200	59,500
TOTALS:	$ 128,300	$ 151,700	$ 183,200

* 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 1971

None

12. SCOPE OF THE PROJECT

A large number of radioactive nuclides are of 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. A large proportion 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 the Massachusetts General Hospital.

14. TECHNICAL PROGRESS IN FY 1971

Construction of the building to house the accelerator was begun on May 1, 1970 by a general contractor under an agreement with the University of California, Los Angeles, which provided the funds, and architectural and engineering supervision. The structure was accepted for occupancy on Feb. 7, 1971. The cyclotron itself was completed by the manufacturer (Cyclotron Corporation, Berkeley, California) and factory tests were observed and accepted on Sept. 18, 1971. The machine was shipped by truck with magnet pole pieces, yoke and dees all assembled. It arrived on Oct. 6,1971 whereupon it was moved into the vault of the Facility for storage during completion of the building. A cyclotron engineer and radiation physicist were added to the staff of the Facility and, together with the electronics engineer, received technical instruction and indoctrination during short stays at the Cyclotron Corporation factory. This basic operating staff, together with personnel of the Laboratory Shops Section undertook actual installation of the cyclotron, with assistance and guidance from the manufacturer's representative. The considerable amount of piping, wiring and mechanical work was essentially complete by mid-March, 1971. Barring unforeseen difficulties acceptance tests should be completed by the end of April, 1971 and external

beams should become available for productive work soon thereafter. A large amount of effort was directed to the procurement, construction, assembly, and testing of ancillary equipment. These included a novel target transport system; a fairly elaborate, safety interlock system; a closed-circuit television monitor system with remotely controlled mirror periscope; gas target handling system; gas analysis equipment, gas chromatograph; solid-state, germanium detector system with 1600 channel analyser for gamma spectrometry; gamma area-monitoring system, continuous air-monitoring system for radioactive particulate and gaseous effluents; and special hoods, shields, etc. for the "hot lab". A detailed safety manual for operations was composed and reviewed by the appropriate campus, State and AEC regulating authorities.

15. EXPECTED RESULTS IN FY 1972

The first effort will be to produce a number of easily produced radionuclides for which clear-cut c linical applications already exist in the Medical Center and other nearby hospitals. As the cyclotron team becomes more proficient an increasing fraction of the effort will turn to generation of nuclides more difficult to obtain, and those which are untried but with some promise of clinical value. The following nuclides will receive first attention: Carbon-11 in the form of carbon monoxide for measurement of blood pools and repetitive red-cell volume determinations; C-11 as carbon dioxide for lung function studies and detection of abnormalities of cardiac structure and function; Nitrogen-13 gas for measuring clearance rates in lung airway diseases; Gallium-67 and Indium-111 for tumor localization studies; Potassium-43 and Cesium-129 for studies of the myocardium and of body electrolyte pool sizes; and Fluorine-18 for bone scanning. These nuclides will also be provided to other groups, such as the Radiodiagnostic Agent Development section of the Laboratory, for use in labeling various chemical agents with radioactive tags. Toward the end of this fiscal year, attempts to prepare Iodine-123 of adequate purity and yield will probably be initiated. Iron-52 will not be produced this year because the Cr target must be mounted inside the cyclotron and we do not yet have the necessary equipment for internal beam irradiations.

During this period, some modest collaborative studies will be undertaken involving the direct bombardment of purified compounds of biologic interest in programs of the Radiobiology Division of the Laboratory.

16. EXPECTED RESULTS IN FY 1973

During this period many of the nuclides mentioned above will become routinely 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 1972 by other groups, such as the Radiodiagnostic Agent Development Section, 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. The production of Oxygen-15 (2 minutes half-life) by deuteron bombardment of nitrogen gas is simple and efficient, but the logistical problems of transporting this gas to the clinical radioisotopes area of the

Department of Radiology in the Medical Center for use in nuclear medicine are serious. Hopefully, they may be solved by installation of a pneumatic-tube delivery system during this year.

Analytical procedures for determining the amounts of various chemical elements in metallurgical samples by radioactivation with charged particles (such as protons, deuterons and helium-3) are coming into wider usage each year. We will explore the potentialities of this technique for samples of biologic origin, particularly those in which the proportion of the stable isotopes of the element of interest has been intentionally altered by administration of an enriched stable isotopic mixture to the organism.

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 - 1971

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. and
Donna L. Vredevoe

8. Project Term: From: 1955 To: Continuing

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	4	3 1/2	2 3/4
(b) Other Tech.	1/2	1/2	1/2
TOTAL:	4 1/2	4	3 1/4

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 52,200	$ 47,000	$ 41,000
(b) Materials and Services	12,600	11,900	10,100
Sub-Total Direct Project Support	$ 64,800	$ 58,900	$ 51,100
(c) Indirect Expenses *	33,200	32,800	34,000
TOTALS:	$ 98,000	$ 91,700	$ 85,100

* 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

Vredevoe, D.L. and Hays, E.F., "Decreased Growth Capacity of Passaged Gross Virus-Induced Mouse Lymphomas After Inoculation With Antithymocytic Sera." Infec. Immun. 2:11-14 (1970).

Frey-Wettstein, M. and Hays, E.F., "Immune Response in Preleukemic Mice." Infec. Immun. 2:398-403 (1970).

Vredevoe, D.L. and Hays, E.F., "Increased Incidence of Lymphoma in C3H/HeJ Adult Mice Injected With Gross Virus and Antithymocytic Serum." Infec. Immun. 2:723-726 (1970).

Vredevoe, D.L., "Isolation and Transplantation of 10-20 Mouse Lymphoma Cells in a Semisolid Medium." Transplantation 10:557-558 (1970).

Hays, E.F., "Lymphoma Development and Karyotype Analyses in Mice With Graft-Versus-Host (GVH) Reactions." Proc. Am. Assn. for Cancer Res. Apr. 1971.

12. SCOPE OF THE PROJECT

During the past year the exciting finding of an RNA dependent DNA polymerase in cancer-inducing RNA viruses, i.e., DNA is synthesized from a viral RNA template, has been published by Howard Temin and confirmed by other groups. This observation has made it imperative to see how this reaction relates to the viral induction of lymphoma in the mouse thymus, a field which has absorbed the interest of this laboratory for several years. We have shown that thymic grafts exposed to Gross lymphomagenic virus (an RNA virus) readily transform to lymphoma. We are currently studying the effects of ionizing radiation and various metabolic inhibitors, among them Rifampin, a known inhibitor of the RNA dependent DNA synthetic enzyme, on lymphoma development in thymic grafts. These studies will tell us how general (irradiation caused) or specific (induced by metabolic inhibitors) alterations in virus-cell interaction can effect the predictable transformation to lymphoma which has been found to occur in these grafts.

Because of the known finding in clinical medicine, that patients with certain diseases associated with alterations in immune reactivity have an increased incidence of neoplasia, combined with reports in the literature showing increased numbers of lymphomas in mice with chronic graft-versus-host reactions (an experimental form of altered immune reactivity), we have undertaken a study to determine the role of lymphomagenic virus and genetic factors in the development of lymphoma in mice with GVH reactions.

The tumor antigens of *in vivo* cloned mouse lymphoma cells are under active study using the ingenious technic developed by Dr. Vredevoe (see publications). The objective of this study is to elucidate those lymphoma cell or host characteristics which favor multiplication of the neoplastic cell or cells in the initial stages of lymphomagenesis--a point which has relevance to the therapeutic consideration of human leukemia and lymphoma. For purposes of the present studies, "characteristics" are operationalized in terms of lymphoma cell virus content or growth potential, and host reactivity to tumor specific antigens. This work focuses on studies of single or low doses of lymphoma cells.

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 mechanisms are being carried out. The work of these investigators is related to that being done 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. Marcell 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 1971

Studies concerned with the effects of direct irradiation of the target organ for mouse viral lymphomagenesis, the thymus, have been completed. AKR mice were thymectomized as young adults and subsequently grafted under the renal capsule with neonatal thymus exposed to Gross virus and 850 or 5000 r of ^{60}Co irradiation. These irradiated grafts regenerated completely within two weeks. When the thymus used for grafting was exposed to virus *in vivo*, then irradiated, or when irradiated just prior to *in vitro* virus exposure, the lymphoma incidence was sharply reduced when compared with that in non-irradiated grafts treated in a similar manner. (The virus itself was not inactivated by 5000 r). However, when virus exposed irradiated thymus was grafted to hosts which had been given neonatal virus inoculations, the lymphoma incidence was the same as that found in non-irradiated control grafts. Irradiation, therefore, does not prevent regeneration of thymic grafts (a function of the radioresistant epithelial reticular cells) and regenerated irradiated thymic grafts in virus-infected hosts regularly develop lymphoma. Irradiation inhibits viral lymphomagenesis in this system, presumably by preventing an important virus-cellular interaction occurring shortly after exposure of the thymus to virus.

Similar experiments using *in vitro* incubation of virus exposed grafts to the metabolic inhibitors, actinomycin D, rifampin, cytosine arabinoside, and puromycin are in progress.

The role of altered host immune reactivity in the development of lymphoma has been the object of intensive scrutiny during the past year. In an attempt to define the role of a lymphomagenic virus in the increased lymphomagenesis observed in mice undergoing graft-versus-host (GVH) reactions, definitive studies have been carried out. They show that lymphoma developing in a situation where a chronic GVH reaction exists is due purely to the presence of virus in the cells inoculated to produce the reaction rather than a state of altered immune reactivity. The GVH reactions are produced by inoculating parental strain spleen cells into F_1 hybrid recipients.

In a hybrid cross where one parent is from a strain with a high natural incidence of reticulum cell sarcoma and the other parent had a marker chromosome, the animals with the GVH reactions had a slightly increased lymphoma incidence and small numbers of cells with the donor karyotype were found in the tumor bearing animals.

In collaboration with Charles G. Craddock, M.D., Professor of Medicine, UCLA School of Medicine, a program has been initiated to study the growth properties and cellular kinetics of bone marrow colonies in vitro. Technics similar to those used by Metcalf and Pike and Robinson have been developed and have been found to promote in vitro colony formation in mouse and human bone marrow. In these studies it is planned to look at marrow from two mouse strains with a high natural incidence of lymphoma, as well as that from normal human beings, and patients with various hematologic disorders.

The effects of immunosuppression on the development of mouse lymphoma have been studied in a system which utilized antithymocytic sera (ATS) as the immunosuppressive agent and Gross virus-induced mouse lymphomas as the lymphoma model. It was shown that ATS, 1) increased the incidence of lymphoma when administered with Gross virus to adult C3H/HeJ mice and, 2) decreased the growth capacity of syngeneic lymphoma cells after cell-ATS incubation in vitro prior to transfer in vivo.

More precise methods for in vitro and in vivo lymphoma cell study were developed. A technic was developed for transfer of single lymphoma cells in a semisolid, nontoxic, optically clear gelatin medium.

15. EXPECTED RESULTS FY 1972

Since we have shown that irradiation inhibits viral lymphomagenesis by preventing an important virus cellular interaction occurring shortly after exposure to virus, the studies with specific metabolic inhibitors will help determine the nature of this interaction. Does it involve RNA directed DNA synthesis? DNA synthesis? Transcription? Protein synthesis, or a combination of these factors? In the course of these studies, we hope to develop a quantitative assay for murine lymphoma virus based on the mixed cytopathology test of Klement, et al., (PNAS 63:753 1969).

The work with bone marrow colonies should show whether the presence of lymphomagenic virus or the prelymphomatous state will alter the colony forming potential of mouse marrow. We have evidence now from radiation protection data that marrow from the high lymphoma AKR strain is deficient in cells that promote recovery after lethal irradiation. In the human marrow studies, we hope to learn more about the nature of the stem cells which form the colonies and their alteration (if such exists) in lymphoma and leukemia.

Single Gross virus-induced mouse lymphoma cells or low doses of such cells will be isolated and transplanted to syngeneic recipients. Three systems will be used: AKR; C3H/HeJ; CBA/H (CBA/T_6T_6 donor lymphoma). The growth potential as measured by incidence and latent period of lymphoma will be determined for syngeneic donor cells of various origins: 1) Gross virus-induced or-accelerated lymphomas, not passaged, 2) passaged lymphomas and 3) spontaneous lymphomas (AKR). Lymphomas maintained in tissue culture may also be studied. The specific aims of the project are:

1. To determine whether there is an association between virus content of lymphoma cells and their growth potential in non-immunized syngeneic recipients.
2. To determine whether differences in host reactivity to tumor-specific antigens and Gross virus alters growth potential of single and low doses of lymphoma cells. AKR mice, frequently reported to be tolerant to Gross virus, will be compared to C3H/HeJ and CBA/H mice, which could be considered to be nontolerant.

3. To determine whether immunization to lymphoma cells or virus of a recipient will result in a decreased incidence of growth of single syngeneic lymphoma cells of known virus content. Specifically, tests will be made to see if there is a correlation between tumor-specific or virus neutralizing antibodies and incidence of lymphoma growth from single or low doses of syngeneic lymphoma cells in the C3H/HeJ mouse system.

During fiscal year 1972, an assay for theta antigen (Reif, A.E., Allen, J.M.V., J. Exp. Med. 120:413-433 1964) will be developed for use with clones of lymphoma cells derived in vivo from transplantation of a single syngeneic cell. If lymphomas can be considered to be developed from thymus-derived cells, theta antigen should be demonstrable on them. However, will all lymphoma cell clones carry such a marker? Will theta antigen content correlate with virus content, growth potential, or tumor specific antigen content?

16. EXPECTED RESULTS FY 1973

We hope by 1973 to have some meaningful results which could tell us more specifically how lymphomagenic virus interacts with thymic cells to produce the change that results in lymphoma.

By this time, we will have completed many studies with marrow from patients with hematologic disorders, as well as, from normal individuals. We will know what factors stimulate in vitro colony formation, the cellular characteristics of the colonies formed, as well as, the ability of the cells to incorporate H^3 Thymidine in vitro. In the mouse, using in vivo labeling of marrow cells, we will be able to follow this label in the in vitro system. This work, combined with studies with marrow depleted of various cellular elements, will lead to a better understanding of marrow stem cells.

Based on results obtained during 1972, further refinements of technics and research designs could lead to answers to the following types of questions:

1. Do single lymphoma cells isolated from hosts with tumors of varying virus content grow at the same rate?
2. Do single lymphoma cells isolated from lymphomatous tissue from one mouse grow at the same rate?
3. Do clones of cells derived from different single lymphoma cells possess different growth characteristics, antigenicity and virus content?
4. Does immunization of hosts harboring a single lymphoma cell result in a lower incidence of lymphoma than in a comparable non-immunized host transplanted with a single lymphoma cell?

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: MAMMALIAN RADIOBIOLOGY

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

4. Date Prepared: April - 1971

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: 1971

9. Man Years:	FY 1971	FY 1972	FY 1973
(a) Scientific	3/4	0	0
(b) Other Tech.	-	0	0
TOTAL:	3/4	0	0

10. Costs:	FY 1971	FY 1972	FY 1973
(a) Direct Salaries	$ 17,300	$ 0	$ 0
(b) Materials and Services	2,400	0	0
Sub-Total Direct Project Support	$ 19,700	$ 0	$ 0
(c) Indirect Expenses *	8,300	0	0
TOTALS:	$ 28,000	$ 0	$ 0

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

11. PUBLICATIONS DURING FY 1971

Hennessy, T. G.: Transferrin Saturation in In-Vitro Erythropoietin Assay. Submitted to Nature.

Okunewick, J. P. and Hennessy, T. G.: Effects of X-Irradiation on Red Cell Carbonic Anhydrase. Submitted to Radiation Research.

M. Frey-Wettstein and C. G. Craddock: Testosterone-Induced depletion of Tymus and Marrow Lymphocytes as Related to Lymphopoiesis and Hematopoiesis. Blood XXXV No. 3 pages 357-271 (Collaborative Program).

12. SCOPE OF THE PROJECT

In the past this project was concerned with elucidating the control mechanisms of red cell production in mammals including man. The particular facets of this general problem that have been studied most recently were the hormonal aspects of erythropoietic control, in particular erythropoietin levels and their reaction to disease states, including radiation damage to bone marrow and the possibility of treatment of this damage with erythropoietin.

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 1971

The study last year which clearly showed that erythropoietin must be administered during the first day after irradiation injury to have therapeutic effect on bone marrow radiation injury was not continued and completed due to lack of funding.

The study of a possible inhibitor of erythropoiesis in the serum of patients with aplastic anemia was completed and the apparent inhibitor shown to be the result of iron saturation of the transferrin in the erythropoietin assay system. The results of this study have been submitted for publication.

The laboratory aspects of this program were discontinued this year due to the lack of funding.

15. EXPECTED RESULTS IN FY 1972 AND FY 1973

Project discontinued.