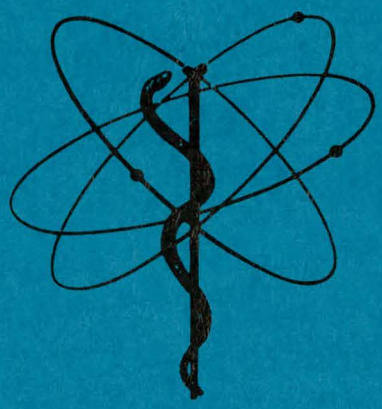
LABORATORY OF NUCLEAR MEDICINE AND RADIATION BIOLOGY

UNIVERSITY OF CALIFORNIA
900 VETERAN AVENUE
LOS ANGELES, CALIFORNIA



AEC CONTRACT AT(04-1) GEN-12

DISCLAIMER

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

MASTER

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

RESEARCH AND DEVELOPMENT PROGRAM
FISCAL YEAR 1974

APRIL 1972

TABLE OF CONTENTS

TABLE OF CONTENTS

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

U. S. Atomic Energy Commission — Biology and Medicine
SAN FRANCISCO OPERATIONS OFFICE — 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.

Decisions on programmatic adjustments made in the last year or two are being implemented and the impact of these adjustments is reflected in the FY 1973 and FY 1974 budget requests. In view of this, brief comments on all of our programs, as organized by divisions, with reference to budgetary needs and changes might help to provide perspective.

Biochemistry: (Budget Category 06 01 02). This high-quality program remains at about the status quo, but the return to AEC funding of two permanent staff members temporarily funded from other sources in FY 1972 will increase costs slightly in FY 1973 and FY 1974.

Developmental Cell Biology: (Budget Category 06 01 02). This relatively new program has not expanded to the level projected when it was undertaken because of budget limitations. Modest expansion of support is required to firmly establish this program, which is highly productive and has exceptional promise for important contributions.

Environmental Radiation: (Budget Category 06 02 04). Top priority has been given in the last two or three years to strengthening the environmental biology program. A most important development during the past year has been the recruitment of Dr. Eric Edney to head up this program. Reorientation of the program is now taking place, but a central part of the strategy is recruitment of two additional staff members to add strength in critical areas. The cost of these recruitments will be partially offset by reductions

in other programs, but an additional $30,000 will still be required over the Congressional Allocation in FY 1973 if we are to proceed with these plans in an orderly fashion.

Nuclear Medicine: (Budget Category 06 03 01). The Biomedical Cyclotron became fully operational in FY 1972. In support of the capability, expanded activity in radiopharmaceuticals and clinical research is taking place. To capitalize on the cyclotron, modest increases in this budget area are required. It is expected that important interaction with the new Radiation Measurement Division will develop, beginning in FY 1973.

Radiation Biology: (Budget Categories 06 01 01 and 06 01 02). This important division in the Laboratory has been understaffed and undersupported in the past due to severe budget stringency. Two additions to the staff have been made on a non-salaried basis with support being mostly in the form of laboratory space. To provide modest levels of support in one of the new areas, Dr. Bennett's late effects program was reoriented into a collaborative program with Dr. Byfield on the effects of ionizing radiation on mammalian animals and tissue. This program is very productive, although minimally supported, and we request a modest increase in FY 1974.. Other areas of Radiation Biology have suffered for insufficiency of technical support which we seek to add in the FY 1974 budget.

Radiation Measurement: (Budget Category 06 02 08). This is a new program initiated this year, and developed by Mr. Huth. Basic research will be conducted in radiation sensing by physical means. Application areas will be in medicine and in the area of environmental problems. These programs will interact importantly with other programs in the Laboratory. It is obviously difficult to project accurately the rate of buildup in the program; however, we expect a more rapid development than would be allowed by the Congressional Budget and are therefore requesting an increase. An additional increase will be needed in FY 1974 to enable this program to achieve its objectives.

As the above comments indicate, many excellent programs have been undersupported in recent years. Nevertheless, even within these limitations important changes have taken place and new programs added to improve the capability of the Laboratory to continue to make important contributions to AEC mission-objectives in Biology and Medicine.

COST INFORMATION:

The composition of costs and staffing for FY 1972, FY 1973, and FY 1974, are summarized below for the entire Biology and Medicine Program by major categories of expense.

	FY 1972	FY 1973	FY 1974
I Costs: (Shown in Thousands)			
Salaries (Direct)	$1,187.6	$1,391.0	$1,631.0
Supplies, Travel, & General Expense	363.4	474.0	529.0
Indirect Costs	790.0	810.0	840.0
Total Operating Costs	$2,341.0	$2,675.0	$3,000.0
II Manpower:			
Direct Man Years	91 ½	103	122
Direct Scientific Man Years	80	91 ½	109

		FY 1972	FY 1973	FY 1974
III	Cost Per Man Year Data: (Shown in Thousands)			
	Cost Per Direct Man Year	$ 25.9	$ 26.0	$ 24.6
	Cost Per Direct Scientific Man Year	$ 29.3	$ 29.2	$ 27.5

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 1972	FY 1973	FY 1974
Composition of Indirect Expense:				
I	Manpower (Shown as Full Time Equivalence):			
	Administrative Services	23	23	24
	Technical Services	6	6	6
	Building Maintenance	7	7	7
	Total Indirect Personnel	36	36	37
II	Costs (Shown in Thousands):			
	Administrative Services	$ 347.4	$ 353.9	$ 376.9
	Technical Services	78.4	80.6	83.1
	Building Maintenance	68.3	69.0	70.4
	Building Amortization	126.7	126.7	126.7
	Utilities	72.1	80.2	83.3
	U. C. Management Allowance	30.0	30.0	30.0
	U. C. Accounting & Purchasing Services	34.6	36.0	36.0
	Miscellaneous (Laundry, Postage, General Supplies, etc.)	32.5	33.6	33.6
	Total Indirect Costs	$ 790.0	$ 810.0	$ 840.0

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 1972 COSTS	FY 1972 MAN YRS	FY 1973 COSTS	FY 1973 MAN YRS	FY 1974 COSTS	FY 1974 MAN YRS
06 01 01	Effects of Radiation on Living Organisms						
	Applied Mammalian Radiobiology (Byfield and Bennett)	$ 26.0	1 1/4	$ 25.0	1	$ 45.0	2
	ACTIVITY TOTAL	$ 26.0	1 1/4	$ 25.0	1	$ 45.0	2
06 01 02	Molecular and Cellular Radiobiology						
	General Metabolism (Mead)	$ 68.1	2 3/4	$ 77.1	3 3/4	$ 77.9	3 1/2
	Organic Chemistry (Howton)	72.5	2 1/4	73.1	2 1/4	77.4	2 1/2
	Tracer Synthesis (Nevenzel)	43.0	2 1/4	53.1	2 1/4	55.8	2 1/2
	Biosynthetic Control (Fulco)	52.7	2 1/4	59.5	2 1/4	58.4	2 1/2
	Developmental Regulation (Harary)	107.0	5	110.7	5	112.9	5
	Developmental Neurobiology (Herschman)	59.1	2 1/2	67.3	2 1/2	81.9	3 1/2
	Mammalian Cell Biology (Gerschenson)	71.3	2 1/2	70.6	2 1/2	92.3	3 1/2
	Developmental Radiobiology (de Vellis)	72.0	3	73.3	3	83.8	4
	Chemical Radiobiology (Myers)	105.7	4	93.6	3 1/2	122.6	4 1/2
	Sub-Cellular Radiobiology (Ward)	67.5	2 3/4	68.0	2 3/4	85.6	3 3/4
	Physical Radiobiology (Strickland)	84.1	3 3/4	78.7	3 1/4	87.4	3 3/4
	ACTIVITY TOTAL	$ 803.0	33	$ 825.0	33	$ 936.0	39
06 02 04	Land and Fresh Water Environmental Sciences						
	Soil Factors (Nishita)	$ 88.8	3 1/4	$ 93.2	3 1/4	$ 106.5	3 3/4
	Environmental Factors (Hawthorne)	73.1	2 3/4	37.0	1 3/4	0	0
	Ecology of the Nevada Test Site (Beatley)	62.0	2 3/4	61.3	2 1/4	72.2	3 3/4
	Vertebrate Radioecology (Turner)	66.3	2 3/4	121.7	4 1/2	140.7	6
	Analysis of Ecosystems (Turner)	42.1	1 3/4	73.3	2 3/4	85.6	3 1/4
	Chemical Problems (Wood)	122.4	5 1/4	119.6	5 1/4	126.6	5 3/4
	Distribution and Interrelationship of Elements in Biological Systems (Alexander)	22.9	1	24.0	1	24.6	1

	Ecology of Desert Arthropods (Edney)	0	0	81.7	2 1/4	83.4	2 3/4
	Nutrient and Radionuclide Cycling (Romney)	0	0	218.2	8 1/2	260.4	11 3/4
	Plant Factors (Romney)	88.5	3 1/4	0	0	0	0
	Plant Physiology Ecology (Wallace)	76.7	1 3/4	0	0	0	0
	Physiology of Mineral Accumulation in Plants (Lunt)	30.6	1	0	0	0	0
	Quantitative Plant Ecology (Wallace)	60.9	2 1/2	0	0	0	0
	Soil Survey and Characterization (Hale)	32.7	1	0	0	0	0
	ACTIVITY TOTAL	$ 767.0	29	$ 830.0	31 1/2	$ 900.0	38
06 02 08	Radiological & Health Physics & Instrumentation						
	Medical Physics Instrumentation (Cassen)	$ 46.0	2	$ 0	0	$ 0	0
	Radiation Measurements Program (Huth)	25.0	1/4	285.0	10	350.0	12
	ACTIVITY TOTAL	$ 71.0	2 1/4	$ 285.0	10	$ 350.0	12
06 03 01	Nuclear Medical Research						
	Clinical Nuclear Medicine (Taplin)	$ 126.6	5 1/4	$ 150.1	6 1/4	$ 146.8	6 1/4
	Basic Nuclear Medicine (Poe)	74.2	3 1/4	83.8	3 1/2	83.8	3 3/4
	Nuclide Metabolism (MacDonald)	63.0	2 1/2	52.9	2 1/4	52.1	2 1/4
	Radiodiagnostic Agent Development (Robinson)	92.0	3 1/2	93.6	3 1/2	134.0	5 3/4
	Clinical Studies: Short-Lived Isotopes (Webber)	52.9	2 1/4	54.5	2 1/2	54.4	2 1/2
	Biomedical Cyclotron Facility (MacDonald)	175.1	5 1/2	205.2	6 3/4	225.4	7 3/4
	Leukemia Biology (Hays and Vredevoe)	90.2	3 3/4	69.9	2 3/4	67.5	2 3/4
	ACTIVITY TOTAL	$ 674.0	26	$ 710.0	27 1/2	$ 769.0	31
	TOTAL 060000 PROGRAM	$ 2,341.0	91 1/2	$2,675.0	103	$3,000.0	122

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 - 1972

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

6. Working Location: UCLA

7. Person in Charge: John E. Byfield and Leslie R. Bennett

8. Project Term: From: 1970 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	1 1/4	1	2
(b) Other Tech.	0	0	0
TOTAL:	1 1/4	1	2

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 15,000	$ 13,000	$ 25,000
(b) Materials and Services	4,600	6,700	7,900
Sub-Total Direct Project Support	$ 19,600	$ 19,700	$ 32,900
(c) Indirect Expenses *	6,400	5,300	12,100
TOTALS:	$ 26,000	$ 25,000	$ 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 1972

Byfield, J. E., and Y. C. Lee: Effects of Cold-Shifts on the Translational Efficiency in Tetrahymena. J. Plant and Cell Physiol. 12, 461-464 (1971).

Draz, S., J. E. Byfield, P. E. Byfield, and E. W. Fonkalsrud: Liver Histiocytes in the Rejection of Tumor Cells. Arch. Surgery 102, 574-577 (1971).

Thompson, R. W., J. E. Byfield, R. Kagan, and E. A. Langdon: Some Recent Advances in Radiation Therapy. Ann. Int. Med. 75 (1), 97-110 (1971).

Draz, S., R. Minami, J. E. Byfield, and E. W. Fonkalsrud: Biochemical Evaluation of Hepatic Preservation Techniques Using the Isolated Rat Liver. Surgery 70, 446-451 (1971).

Byfield, J. E.: Are the Genes Controlling Cytokinesis Late-Replicating and Located on the X-Chromosome? Europ. J. Clin. Biol. Res. (In Press).

Byfield, J. E.: Ionizing Radiation and Vincristine: Possible Neurotoxic Synergism. Radiol. Clin. Biol. 41, 129-138 (1972).

Byfield, J. E., Y. C. Lee, and L. R. Bennett: A Direct Radioassay for In Vitro Radiosensitization by Chemotherapeutic Agents. Radiol. (In Press).

Finklestein, J. Z., J. E. Byfield, K. Tittle, and D. T. Imagawa: Studies of Inhibition of Tumor Cell DNA Synthesis by Immune Cells in Gross Virus Induced Leukemia. Europ. J. Clin. Biol. Res. (In Press).

Draz, S., J. E. Byfield, and E. W. Fonkalsrud: Hepatic and Splenic Histiocyte Stimulation of Phagocytosis of Syngeneic Tumor Cells. Surg. For. 22, 111-112 (1971).

Byfield, J. E. and U. Karlsson: Induction of Neuroblastoma Differentiation by Cytosine Arabinoside. (Submitted).

Lee, Y. C., J. E. Byfield, and L. R. Bennett: Quantitative Aspects of Radiation Repair in Mouse L-1210 Leukemia Cells. (Submitted).

Byfield, J. E., I. Klisak, and L. R. Bennett: Thermal Bone Marrow Expansion. (Submitted).

Byfield, J. E., Y. C. Lee, and L. R. Bennett: An In Vitro Study of Radiosensitization in Human Cancer Biopsies. 2nd Cong. Europ. Assoc. Radiol., Amsterdam, 1971, Excerpta Medica Intern. Cong. Ser. 230, 106 (1971) (Abstract).

Byfield, J. E. and U. L. Karlsson: Induction of Neuroblastoma Differentiation by Cytosine Arabinoside. Demonstration at Xth International Conference on Embryology, Glasgow, 1971 (Abstract).

Byfield, J. E., Y. C. Lee, and L. R. Bennett: Radiation Repair in Mouse L-1210 Leukemia Cells. Amer. Soc. Cell Biol., New Orleans, November, 1971, p. 42 (Abstract).

Byfield, J. E., and U. L. Karlsson: Induction of Neuroblastoma Differentiation by Cytosine Arabinoside. Amer. Zool. 11, 687 (1971), presented at 1971 meeting Amer. Assoc. Adv. Science, Philadelphia, December, 1971 (Abstract).

Byfield, J. E., L. R. Bennett, and A. R. Kagan: Thermal Bone Marrow Expansion. Proc. Amer. Assoc. Ca. Res. (In Press) (Abstract).

Lee, Y. C., J. E. Byfield, P. Chan, and L. R. Bennett: Inhibition of Radiation Repair in L-1210 Cells. Proc. Amer. Assoc. Ca. Res. (In Press) (Abstrac

Byfield, J. E., Y. C. Lee, and L. R. Bennett: Evaluation of Single Strand Repair Inhibitors (Type III Radiosynergizers) In Vitro. Rad. Res. (In Press) (Abstract).

Lee, Y. C., L. R. Bennett, and J. E. Byfield: Inhibition of Single Strand Breaks by Antibiotics and a Normal Metabolite. Rad. Res. (In Press) (Abstract).

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 being pursued in this project relates closely to other investigations in this division which deal with the mechanisms of radiation damage. However, we are particularly interested in examining in detail the process of repair of X-ray damage in mammalian cells, and particularly the effects of various drugs on inhibiting this repair. In other projects being conducted here we are interested in developing means of increasing radioprotection (and protection against radiomimetic drugs) through a process we term "thermal bone marrow expansion." This work necessarily relates to studies being conducted in several of the AEC national laboratories and to many hematological and radiobiological studies being done throughout the world.

In other work we are interested in the developmental biology of neuroblastoma cells, particularly in the reversibility of the malignancy of these cells. Specifically, we are concerned with the tumor-specific transplantation antigens of these cells and the role of murine C-type viruses in the malignant transformation of murine neuroblasts. These studies parallel but do not overlap several other investigations within the Developmental Biology Division of the Laboratory, particularly the work of Drs. Herschman and de Vellis.

Finally, we are continuing our work on analysis of the life cycle of eukaryotic cells. This particular investigation is in transition. We have almost completed the studies on synchronized Tetrahymena and will begin analogous experiments on mammalian cells during the next fiscal year. These studies relate closely to the work on life-cycle analysis being pursued at several other AEC installations particularly Los Alamos and Argonne.

14. TECHNICAL PROGRESS IN FY 1972

Thermal Bone Marrow Expansion: It was shown in 1936 by Huggins and Blocksom that implantation of rat tail or limb bones into the peritoneal cavity led to red bone marrow formation (J. Exptl. Med. 64, 253). The increasing use of both total nodal irradiation and numerous cycle-active drugs in cancer therapy prompted us to re-evaluate this phenomenon in the hope that significant marrow expansion could be induced by elevation of the ambient temperature. We used adult Swiss mice and determined the degree of marrow increase by histological examination and by net uptake of ^{59}Fe (amputating the tails). Distribution of marrow was determined by ^{59}Fe autoradiography. Ambient temperature was manipulated by using a tissue culture incubator intermittently perfused with air to reduce moisture accumulation which was otherwise highly lethal. We report the following: (a) Huggins' and Blocksom's hypothesis that it is temperature per se that allows marrow differentiation is confirmed and extended to another species; (b) marrow expansion begins a few days after elevation and is maximum by one month; (c) the rate of expansion is roughly a function of the ambient temperature between room and body temperature; (d) pre-existing anemia stimulates expansion; (e) both ^{59}Fe and ^{99m}Tc are useful in following this process and evaluating secondary stimuli such as hormones.

Studies on Neuroblastoma Differentiation: Murine neuroblastoma cells can be induced to differentiate in culture by exposure to cytosine arabinoside at concentrations which do not significantly affect the differentiating fraction. The differentiated cells exhibit the morphological characteristics of sympathetic neurons and show intercellular contacts. They can remain differentiated in culture for at least four weeks but the process is reversible throughout the exposure. Upon removal of the cytosine arabinoside from the medium the cells revert to the undifferentiated form capable of unrestricted growth in culture and tumor formation in syngeneic mice. Tracer studies show that the cytosine arabinoside inhibits only DNA synthesis, the incorporation of RNA and protein precursors being unaffected. A cytoplasmic virus persists throughout these transitions. The experiments illustrate that morphological, and probably physiological, criteria are insufficient to exclude malignant potential in mammalian cells.

Syngeneic A/J mice show a weak immune response against the C-1300 neuroblastoma. This response includes the induction of complement-fixing antibodies and of lymphocytes capable of in vitro cytotoxicity. When immune lymphocytes are exposed to the differentiated form of these cells, cytoxicity persists indicating that tumor specific transplantation antigens are retained on the neuroblastoma cell surface despite differentiation.

Repair of Single Strand Breaks in Mammalian Cells: We have studied the repair of single strand DNA breaks using the modified alkaline sucrose gradient method of McBurney, Graham, and Whitmore (Biochem. Biophys. Res. Comm. 44, 171 (1971) and pers. comm.). Our results confirm McBurney et al., indicating this method to be exceedingly sensitive in detecting single strand breaks. Doses as low as 200 rads yield significant reductions in molecular weight consistent with multiple single strand ruptures. These breaks are repaired very rapidly by the intact cell, repair being initiated immediately and being completed within 60 minutes. Repair of these strand breaks is markedly inhibited by Actinomycin D, the inhibition being an inverse function of time and a direct function of drug concentration. Most pertinently, inhibition of single strand repair can be shown to occur at Actinomycin D levels which approximate the concentration to be expected in vivo during

therapy of human tumors (e.g., 0.028 ug/ml yields significant delay in strand rejoining). The data strongly imply that combined radiation and drug therapy is a rational approach to improving cancer therapy. However, the rapidity of repair and the lack of effect of several antimetabolites indicate that selection and clinical usage of this type of radiosynergism can be improved. For example, we have also found that at least one normal metabolite, DL-glyceraldehyde, is a strong inhibitor of single strand break repair. Neither drug screening nor time/(drug: radiation) dose fractionation for this type of radiosynergizer is probably optimal as currently practiced, based on the data obtained thus far.

Development of Improved Screening Methods for Radiosynergizing Drugs: Cancer chemotherapeutic agents can increase cellular sensitivity to ionizing radiation by rendering DNA strands more liable to ruptures (type I), by inducing cell cycling (type II), and by inhibiting repair of sub-lethal damage (type III). In this laboratory we have been studying the type III radiosynergizers with a view to developing an improved screen for such drugs and for determining in vitro which drugs may be most effective against specific cell lines including biopsies. Our approach exploits Cleaver's original observation that certain drugs (hydroxyurea, cytosine arabinoside) block normal semi-conservative replication but do not block repair replication. This suggested that net post-irradiation thymidine incorporation by cytosine arabinoside treated cells would directly monitor inhibition of repair replication. We then studied this phenomenon in detail in mouse L-1210 leukemia cells using the alkaline CsCl method. The data were quite consistent with the hypothesis and suggested this approach was feasible (Byfield et al., Radiol., submitted). However, high radiation doses (100 Kr) were required. We have now studied the effect of low doses of radiation (200-1,000 rads) using the modified alkaline sucrose gradient method of McBurney, Graham, and Whitmore. The results show that cytosine arabinoside concentrations (7 ug/ml) which block normal DNA synthesis have no effect on single strand repair while low concentrations of added second drugs (e.g., actinomycin D) inhibit both single strand repair and thymidine incorporation. The data strongly imply that in vitro radiosensitizers can be tested and that a more explicit screen for type III radiosynergizers might be profitable for clinical treatment.

Control of Cytokinesis in Eukaryotes: Our studies to date give direct evidence supporting the following hypotheses: (a) the replication sequence dictates the S-G_2-M phases of the cell cycle; (b) a late-replicating DNA fragment exists whose replication is a sine que non for cytokinesis; (c) transcription of this fraction occurs only following its replication and is again blocked by the completion of cell division; (d) this fragment occurs on a late-replicating portion of the X-chromosome in mammals; (e) interaction with this fragment may be a signficant event in oncogenic transformation. These proposals are discussed in detail in the 5th reference above.

15. EXPECTED RESULTS IN FY 1973

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 the following studies:

(a) Effect of Bleomycin on Cellular DNA: This newly available antibiotic is a strand breaker in vitro but apparently slightly differs in its mode

of action from X-rays. We are comparing the two in vivo and in vitro with a view to the mechanisms of action and possible variances in the effects of added second drugs on repair processes in each system.

(b) Studies on Human Polynucleotide Ligase: The most recent data on radiation repair following ionizing radiation suggests that the terminal enzyme in the repair process (polynucleotide ligase) may be critical in clinical dose range repair. At this time no inhibitors of this enzyme are known. A specific project under the direction of Dr. Y. C. Lee will study this enzyme isolated from human cancer cells and will attempt to identify inhibitors of this enzyme.

(c) Thermal Bone Marrow Expansion: We hope to be able to initiate clinical trials of this method later this coming year. In addition, we plan to try to identify the precursor cells in the bone marrow responsible for this effect and also to determine whether this process may have any use in immunologic studies (since it holds the theoretical possibility of developing a syngeneic T-B cell chimaeric state in vivo).

(d) Studies on Human Tumor-Specific Transplantation Antigens: We have initiated a quantitative prospective study designed to determine the possible role of autochthonous immunity in the radiation cure of carcinoma of the cervix - these studies will be continued.

16. EXPECTED RESULTS IN FY 1974

We anticipate that by FY 1974 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: GENERAL METABOLISM

3. AEC Budget Activity No.: 06 01 02

4. Date Prepared: April - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	2	3	3
(b) Other Tech.	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{1}{2}$
TOTAL:	$2\frac{3}{4}$	$3\frac{3}{4}$	$3\frac{1}{2}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 35,700	$ 43,000	$ 45,000
(b) Materials and Services	8,700	8,700	9,400
Sub-Total Direct Project Support	$ 44,400	$ 51,700	$ 54,400
(c) Indirect Expenses *	23,700	25,400	23,500
TOTALS:	$ 68,100	$ 77,100	$ 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 DURING FY 1972

G. A. Dhopeshwarkar, Carole Subramanian and J. F. Mead. Rapid Uptake of 1-^{14}C Acetate by the Adult Rat Brain 15 Seconds After Carotid Injection. Biochim. Biophys. Acta 248, 41-7 (1971).

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J. F. Mead and Roberta Hare. Alpha Oxidation of Cerebronic Acid in Brains from Scorbutic and Ascorbic Acid-Supplemented Guinea Pigs. Biochem. Biophys. Res. Comm. 45, 1451-56 (1971).

F. Wolfgram, Mona E. Fewster and J. F. Mead. Lipids and Amino Acids of Multiple Sclerosis Myelin. Symposium on Selected Topics in Human Chemical Neuropathology. Rivista Di Patologia Nervea E. Mentale, via S. Salvi, 12, Firenze, Italy.

J. F. Mead. Dietary Polyunsaturated Fatty Acids as Potential Toxic Factors. Chemical Technology.

G. A. Dhopeshwarkar, Carole Subramanian, D. H. McConnell and J. F. Mead. Fatty Acid Transport into the Brain. Biochim. Biophys. Acta 255, 572-79 (1972).

Vida Slawson and J. F. Mead. Stability of Unsaturated Methyl Esters of Fatty Acids on Surfaces. J. Lipid Res. 13, 143-46 (1972).

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 metabolically self-contained inert tissues as the brain will it be possible to assess the initial changes occurring with aging and low-level irradiation. The incorporation of lipids into the brain and factors influencing this process will be studied.

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 (Developmental Biology), Dr. D. R. Howton (Organic Chemistry), Jr. J. C. Nevenzel (Tracer Synthesis), Dr. H. Herschman (Developmental Biology), Dr. J. de Vellis (Developmental Biology)

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 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. 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 (Dr. J. Clement, Dr. N. Baumann), Germany (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 1972

In a study of the brain alpha-oxidation system for long-chain fatty acids, the requirements were shown to be: microsomes, 100,000xg supernatant and ferrous ion. The supernatant factor has now been found to be ascorbic acid stabilized by a thiol-containing peptide. The major function of the ascorbic acid appears to lie in maintaining the iron in the +2 state. Guinea pigs on an ascorbic acid-free diet quickly lose the ability to carry out alpha oxidation. The loss can be restored with ascorbic acid in vivo or in vitro and is shown to stem from the rapid decrease in ascorbic acid in the 100,000xg supernatant, whereas the microsomes appear to be normal.

A series of experiments has revealed that fatty acids are incorporated very rapidly into the brain lipids from the blood (within 15 secs. for palmitate). Rapid and extensive incorporation was also observed if the liver was removed from the circulation. However, injected lecithin was not readily incorporated into the brain lipids. These data are in agreement with the hypothesis that no blood-brain barrier exists for fatty acids but that liver competes favorably with brain for the fatty acids converting them into glycerides, which are then unavailable to brain, since it has no lipoprotein lipase.

15. EXPECTED RESULTS IN 1973

During the year, the elucidation of the mechanism of alpha-oxidation should include information on the function of the iron and, possibly, the mechanism of the reaction.

The influence of certain external factors on the passage of fatty acids from blood to brain should be elucidated and the turnover of the brain lipids investigated.

The control of membrane lipid synthesis involving desaturation of fatty acids and incorporation of the unsaturated fatty acids into the lipids of growing membranes should be investigated in hepatocytes in culture.

16. EXPECTED RESULTS IN FY 1974

Future research will naturally depend on the results obtained from current efforts. Nevertheless, it can be anticipated that by the end of fiscal 1974, 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 - 1972
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 1972	FY 1973	FY 1974
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$
TOTAL:	$2\frac{1}{4}$	$2\frac{1}{4}$	$2\frac{1}{2}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 39,600	$ 41,000	$ 45,000
(b) Materials and Services	9,000	8,500	8,900
Sub-Total Direct Project Support	$ 48,600	$ 49,500	$ 53,900
(c) Indirect Expenses *	23,900	23,600	23,500
TOTALS:	$ 72,500	$ 73,100	$ 77,400

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

11. PUBLICATIONS DURING FY 1972

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 infrared, ultraviolet, and mass spectrometric and by unequivocal degradative techniques.

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 1972

Consideration of the fact that carboxylic (fatty) acids are virtually completely dimerically associated in condensed states via hydrogen bonding of their carboxyl groups, together with the apparent likelihood that loss of an electron ("ionization") from such substances should result, with high probability, in localization of the resulting "electron hole" on the carbonylic oxygen atom of the carboxyl group led us to propose [Howton and Wu, J. Am. Chem. Soc. 89, 523 (1967)] that decarboxylation resulting from exposure of fatty acids to ionizing radiation might involve a mechanism curious in that carbon dioxide is lost from the "intact" molecule hydrogen bonded to that from which the electron is actually ejected.

It occurred to us that it might be possible to demonstrate the involvement of such a "crossed" decarboxylation reaction by determination of the relative amounts of two hydrocarbons produced by loss of carbon dioxide from cross-paired mixtures of different fatty acids. Although an equilibrated liquid equimolar mixture of two acids A and B would be expected to contain 50 mole-% of the cross-paired species A...B and thus represent a suitable system, the fact that certain fatty acids (e.g., palmitic and stearic) can be induced to crystallize as a "compound", consisting entirely of cross-paired molecules, provides a more attractive system - particularly if the differences sought proved to be subtle.

In terms of the number of valence electrons Z_i present in the ith component of a mixture and the fraction G_i of the energy absorbed by that component which leads to its decarboxylation (G_i is equivalent to the "G-yield" of decarboxylation products — CO_2 or hydrocarbon AH arising from acid ACOOH - when a single acid is involved and at low radiation dose, where products absorb an inappreciable proportion of the energy and the quantity of product is insignificantly reduced by secondary reaction), it is clear that the mole ratio, C_{15}/C_{17}, of pentadecane (from palmitic acid, P) to heptadecane (from stearic acid, S) will, in the case of an equimolar mixture of P and S in which the two acids are self-paired (i.e., consisting solely of P...P and S...S "dimers"), be equal to G_PZ_P/G_SZ_S. On the other hand, irradiation of the "compound", consisting of an equimolar mixture of P and S which is completely cross-paired (i.e., all P...S) should, if decarboxylation occurs exclusively via the proposed "cross-reaction" mechanism, give rise to a C_{15}/C_{17} ratio of G_SZ_S/G_PZ_P - the inverse of that expected from the self-paired system - since, for example, energy absorbed by S in P...S leads to formation of C_{15} instead of C_{17}, which would have been produced from S...S. (It is well-established that for homologous normal fatty acids, G_i is inversely related to Z_i; fortunately, however, from the standpoint of the applicability of the proposed test, $G_i \neq K/Z_i$, and thus $G_1Z_1/G_2Z_2 \neq G_2Z_2/G_1Z_1$.)

Comparison of products obtained from a thorough admixture of equimolar amounts of finely-divided crystalline palmitic and stearic acids (not ground together, avoiding any possibility of local melting and thus of pair scrambling) with those from a sample of the P-S "compound" (both exposed to 93 Mrads of Co^{60} γ-radiation, with thermostatting to prevent melting during the irradiation) indeed revealed significant differences in the C_{15}/C_{17} ratio in the sense expected on the basis of the involvement of cross-reaction: self-paired, 1.059; cross paired, 0.962 (both $\pm$ 0.005). To obviate secondary-reaction complications in quantitative interpretation of results, the experiment was repeated at a dosage of 1 Mrad (close to the lower limit of precise gas-chromatographic determination of the quantities of hydrocarbons obtained). Here again, the ratios were reversed: self-paired, 0.955 $\pm$ 0.004; cross-paired, 1.035 $\pm$ 0.004. (The interesting reversal of order, e.g., $C_{15}/C_{17} > 1$ for self-paired material exposed to a 93-Mrad dose, and < 1 at 1 Mrad, is understandable in terms of expected secondary reactions.)

Determination of the yield of heptadecane from stearic acid (dose: 1 Mrad) provided the following values ($G_{C_{17}}$): duplicate runs on solidified melts: 3.54 $\pm$ .01 and 3.52 $\pm$ .01; run on compact, finely powdered sample, 3.50 $\pm$ .02 (standard deviation of quadruplicate GLC runs). These results (others involving pure palmitic acid, which will also permit an independent determination of the G_PZ_P/G_SZ_S ratio, are in progress) show that the observed differences in C_{15}/C_{17} ratios from self- and cross-paired mixtures are considerably greater than can be attributed to experimental error or to unknown effects of sample density. Verification of the crucial nature of the P-S "compound"

is also being checked by X-ray diffraction studies supervised by Prof. David Eisenberg (Chemistry Department).

. . . . .

Identification of stearaldehyde as an important product of stearic acid analysis, previously based on gas-chromatographic behavior indistinguishable from that of an authentic sample, has now been confirmed by mass-spectrometric evidence provided by the newly-installed Finnegan 3000 GLC-MS apparatus.

15. EXPECTED RESULTS IN FY 1973

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 1974

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$
TOTAL:	$2\frac{1}{4}$	$2\frac{1}{4}$	$2\frac{1}{2}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 21,400	$ 29,000	$ 32,000
(b) Materials and Services	5,800	6,700	7,000
Sub-Total Direct Project Support	$ 27,200	$ 35,700	$ 39,000
(c) Indirect Expenses *	15,800	17,400	16,800
TOTALS:	$ 43,000	$ 53,100	$ 55,800

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

11. PUBLICATIONS DURING FY 1972

M. Kayama, Y. Tsuchiya and J. C. Nevenzel. The Glyceryl Ethers of Some Shark Liver Oils. Nippon Suisan Gakkaishi [Bull. Japan. Soc. Sci. Fisheries] 37, 111-18 (1971).

R. F. Lee, J. C. Nevenzel and G.-A. Paffenhöfer. Importance of Wax Esters and Other Lipids in the Marine Food Chain: Phytoplankton and Copepods. Marine Biol. 9, 99-108 (1971).

E. A. Baker, J. C. Nevenzel and A. A. Benson. Wax Ester Lipases of Marine Animals. J. Am. Oil Chemists' Soc. 48 [Abstract No. 5], 85A (1971). R. F. Lee, J. Hirota, J. C. Nevenzel, A. R. Lewis and A. A. Benson. Wax Ester Metabolism During the Development of Copepods. Ibid. [Abstract No. 6].

R. F. Lee, J. C. Nevenzel, J. Hirota and A. A. Benson. Wax Esters: Reserve Lipids in Marine Copepods. Fed Proc. 30, 1276Abs [Abstract No. 1304] (1971).

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 1972

The investigation of the lipids of gonostomatid fishes has been completed, and a manuscript is now in preparation. The determination of the structures of the wax ester alcohols and fatty acids was instructive, although we did not isolate any polyunsaturated alcohols. A major component which we had identified on the basis of its retention time in gas chromatography as a C_{20} alcohol containing four double bonds, on isolation by preparative gas chromatography, reductive ozonolysis of the double bond(s), and identification of the fragments by gas chromatography was found to be a mixture of C_{22} monoenes with the (single) double bond at either carbon number 11 (Δ^{11}) or carbon number 13 (Δ^{13}). The structures of the monoenes, both alcohols and acids, proved most interesting. In these marine animals the desaturating enzyme which introduces the first double bond between the 9th and 10th carbon atoms to form the Δ^9 fatty acids acts effectively on the C_{20} and C_{22} homologs, in contrast to the desaturases active in higher terrestial animal and plant species, where the enzyme has a high specificity for the C_{16} and C_{18} homologs, but apparently does not act on longer acids. In Cyclothone acclinidens wax esters, for example, over 80% of the 20:1 alcohols have the double bond at Δ^9 and less than 20% at Δ^{11}, the position expected on the basis of chain elongation of oleic acid (18:1-Δ^9) to the 20:1-Δ^{11} fatty acid and reduction to the corresponding alcohol. Similarly, about 20% of the 22:1 fatty acid was found to be the Δ^9-isomer, presumably formed by direct desaturation of the saturated C_{20} acid.

This basic difference between terrestial and marine organisms may, in fact, lie at the level of the copepods on which the fish feed, as indicated by some work growing out of a cruise last summer in the Straight of Georgia and into Bute Inlet, British Columbia, Canada. We were gathering evidence in support of our hypothesis that the waxy material sometimes found floating on the water's surface in Bute Inlet is derived from zooplankton in this inlet. Bute Inlet Wax contains 50% of wax esters whose alcohol and acid compositions show some resemblances to those of the most abundant copepod in these waters, Calanus plumchrus. The structures of the mono-unsaturated alcohols from the wax esters of C. plumchrus and of the Bute Inlet Wax also showed convincing correlations. In both more than 80% of the C_{20} monoenes were the Δ^{11}-isomers and 22:1-Δ^{11} was the major C_{22} isomer.

Another bonus of the cruise was the finding that the gonads of the sea anemone, Metridium senile (orange variety), contained major amounts of

C_{28}-C_{42} wax esters, while other tissues contained none.

15. EXPECTED RESULTS IN FY 1973

The specific direction our work will take in FY 73 will in part be determined by personnel and budget. Assuming that Dr. Tibor Farkas of the Biological Research Center of the Hungarian Academy of Sciences joins us for a year, we expect to study the lipid metabolism of aquatic organisms at the subcellular or cell-free level. The ultimate objective is to understand in detail the mechanisms controlling wax ester biosynthesis and to establish the specific points of difference between those species which make wax esters and those which make triglycerides.

Hopefully we can also continue to investigate the structures of unsaturated alcohols and acids from additional marine organisms. In a short term study we will survey crustaceans for the occurrence of desmosterol. The lipid patterns of the edible decapoda examined so far (a crab and two shrimp samples) are unusual, and we expect to complete a detailed analysis of at least one species. Total lipid content is low (1-3%, dry wt), the neutral, reserve lipid types are low (but traces of wax esters do occur), and the amounts of total sterols (largely cholesterol, with traces of desmosterol) and phospholipids are relatively high.

16. EXPECTED RESULTS IN FY 1974

The study of lipid metabolism at the subcellular level in aquatic organisms will continue, possibly in association with laboratory culture of suitable species of marine algae, protozoans or copepods. We will determine whether some of the algae and protozoans do biosynthesize wax esters. The inshore, shallow water species of marine copepods which are amenable to mass culture in the laboratory contain only traces of wax esters at most, but they could serve as reference species with which to develop techniques for studying lipid metabolism in cell-free preparations. Such techniques might then be applied at sea to marine bathypelagic copepods or fishes rich in wax esters.

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.:	4. Date Prepared:
06 01 02	April - 1972
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 1972	FY 1973	FY 1974
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{2}$
TOTAL:	$2\frac{1}{4}$	$2\frac{1}{4}$	$2\frac{1}{2}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 30,100	$ 33,000	$ 34,000
(b) Materials and Services	6,800	6,700	6,800
Sub-Total Direct Project Support	$ 36,900	$ 39,700	$ 40,800
(c) Indirect Expenses *	15,800	19,800	17,600
TOTALS:	$ 52,700	$ 59,500	$ 58,400

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

11. PUBLICATIONS DURING FY 1972

J. F. Quint and A. J. Fulco. Substrate Specificities for Fatty Acid Desaturation in Bacilli. Fed. Proc. 30, No. 3, 1079 Abs. (1971).

A. J. Fulco. The Biosynthesis of Unsaturated Fatty Acids by Bacilli. III. Uptake and Utilization of Exogenous Palmitate, J. Biol. Chem. (in press, May, 1972).

A. J. Fulco. The Biosynthesis of Unsaturated Fatty Acids by Bacilli. IV. Temperature-Mediated Control Mechanisms, J. Biol. Chem. (in press, May, 1972).

12. SCOPE OF THE 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 1972

The factors affecting the uptake, utilization and desaturation of exogenous palmitate at constant temperature by Bacillus megaterium 14581 were elucidated and the validity of the in vivo assay for determining the relative levels of the temperature-induced Δ^5-desaturating enzyme in this organism was established. It was found that the presence or absence of glucose in the medium profoundly affected unsaturated fatty acid biosynthesis. In the presence of glucose, palmitate-1-C^{14} is rapidly and completely taken up by the cells but part of the label is excreted within 15 min. as β-oxidation products. The remaining palmitate (30-50% of the added dose) is now completely stable to β-oxidation and becomes part of a readily desaturated endogenous pool (35 mμmoles per g. cells) which remains constant during incubation. In the absence of glucose, the amount of palmitate β-oxidized is doubled, the endogenous pool of palmitate available for desaturation drops to less than 1 mμmole per g. cells and the rate of desaturation decreases by about 20 fold. Results of this kind suggest that neither

palmityl CoA nor the palmitate of neutral or phospholipids are substrates for desaturation but rather the substrate is derived from fatty acid biosynthesis (presumably palmityl ACP). Exogenously derived palmitate is thought to enter this pool indirectly by first being incorporated into a small, metabolically active pool of lipids and then exchanging rapidly with endogenous palmityl ACP. The 4 distinct temperature control mechanisms affecting unsaturated fatty acid biosynthesis (desaturase induction, irreversible enzyme inactivation, reversible desaturase inactivation and decay of the desaturase synthesis system) have now been integrated and equations have been derived which allow one to calculate, as a function of time, the individual contributions of each of these temperature-dependent processes in regulating the level of unsaturated fatty acid biosynthesis at a given temperature in B. megaterium.

We have also prepared 200 mg. of very pure acyl carrier protein (ACP) from E. coli and are presently synthesizing palmityl-1-C^{14}-ACP for testing in cell-free desaturation systems from B. megaterium.

Additional progress was made in work designed to determine the factors which control substrate specificity and positional specificity of double-bond insertion in Bacilli desaturating systems. We have shown, for example, that specificity for chain length is not related to the position of double bond insertion since examples of Δ^5-desaturating systems have been found in various Bacilli which are most active with the C_{16} chain length followed by C_{17} and finally C_{18}, those with exactly the opposite specificity (i.e, C_{18} C_{17} C_{16}) and one desaturase which shows little chain length specificity at all (i.e., $C_{16} = C_{17} = C_{18}$).

15. EXPECTED RESULTS IN FY 1973

1) Complete the synthesis of palmityl-1-C^{14}-ACP and use this substrate to test for desaturation activity in cell-free preparations from Bacilli and to look, as well for an exchange reaction between palmityl-ACP and the palmitate of neutral or phospholipids.

2) Continue to study the effects of chain length, branching, and pre-existing double bonds on the specificity of desaturation in various Bacilli to determine if there is a relationship between specificity of desaturation and the temperature stability of the desaturase enzymes.

3) Elucidate the mechanism of low-temperature induction of Δ^5-desaturase in B. megaterium; that is, to determine what biochemical steps are involved in "turning on" desaturase synthesis when cultures are transferred from 35° to 20°.

16. EXPECTED RESULTS IN FY 1974

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-in sensitive (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 - 1972
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 1972	FY 1973	FY 1974
(a) Scientific	4	4	4
(b) Other Tech.	1	1	1
TOTAL:	5	5	5

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 50,200	$ 57,000	$ 59,000
(b) Materials and Services	17,300	19,500	23,700
Sub-Total Direct Project Support	$ 67,500	$ 76,500	$ 82,700
(c) Indirect Expenses *	39,500	34,200	30,200
TOTALS:	$107,000	$ 110,700	$ 112,900

* 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 1972

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., 142, 501-507 (1971)

Desmond, W. and I. Harary. In Vitro Studies of Beating Heart Cells in Culture: XV Myosin Turnover and the Effect of Serum. Arch. Biochem. Biophys. (Submitted) (1972).

Harary I. Preparations of Rat Heart Cells Methods in Enzymology. Acad. Press (1972) (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 of 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, Kornigsberg - Development of Skeletal Muscle Cells In Vitro Systems; W. Mommaerts - The Role of Ennervation 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; S. Haywood, A. Rich - In vitro synthesis of myosin.

14. TECHNICAL PROGRESS IN FY 1972

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 we have determined the rate of synthesis and degradation in the heart cells under various experimmental

systems. Serum is necessary for the synthesis of myosin and also has factors which protect against its degradation.

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 nonprotein. Proteases do not destroy its activity.

Further analysis of the role of the boiled serum factor indicates that its effect is confined to the stimulation of myosin synthesis. It has no effect on the degradation rate. Furthermore the boiled serum factor stimulates uptake of radioactive amino acids 2 to 3 times more into myosin as compared to the rest of the protein. Nor does it have an effect on level of one general protein, lactic dehydrogenase, or another heart specific protein, creatine phosphokinase. The heat stable factor seems to have its effect on the synthesis of myosin messenger RNA since actinomycin D eliminates its effect on myosin synthesis. In order to investigate the role of this factor in other myosin synthesizing systems we have established rat skeletal muscle cells in culture. In contrast to the heart cells the skeletal muscle cells do not require serum for the synthesis of myosin. These cells start their synthesis of myosin after fusion of the cells and the rate of accumulation of myosin is proportional to growth rather than the presence of serum.

We are continuing our study of the role of cell division and serum in the development of immature heart cells into functioning mysoin synthesizing 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 1973

We will continue to study the development of muscle cells in culture. Since the heat stable factor is specific for myosin synthesis we will use this effect to increase the synthesis of mRNA in polysomes that are synthesizing myosin so that we can more successfully isolate myosin mRNA. In this way we will have a means of studying the conditions which control the initiation and synthesis of myosin and the mRNA for myosin. We will continue to study the nature of the myosin heat stable serum factor.

16. EXPECTED RESULTS IN FY 1974

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.:	4. Date Prepared:
06 01 02	April - 1972
5. Method of Reporting: Publications, UCLA Reports Semi-annual and Final Reports	6. Working Location: UCLA
7. Person in Charge: Harvey R. Herschman	8. Project Term: From: 1970 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2	2	3
(b) Other Tech.	½	½	½
TOTAL:	2 ½	2 ½	3 ½

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 30,900	$ 34,000	$ 43,000
(b) Materials and Services	12,400	13,200	17,100
Sub-Total Direct Project Support	$ 43,300	$ 47,200	$ 60,100
(c) Indirect Expenses *	15,800	20,100	21,800
TOTALS:	$ 59,100	$ 67,300	$ 81,900

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

11. PUBLICATIONS DURING FY 1972

Hershcman, H. R., L. Levine and J. de Vellis, Appearance of a Brain Specific Antigen (S-100 Protein) in the Developing Rat Brain. J. Neurochem. 18, 629-633 (1971).

Cotman, C., H. R., Herschman, and D. Taylor, Subcellular Fractionation of Cultured Glial Cells. J. Neurobiol. 2, 169-180 (1971).

Lerner, M. P., F. O. Wettstein, H. R. Herschman, J. G. Stevens and B. R. Fridlander. Distribution of Polysomes in Mouse Brain Tissue. J. Neurochem. Vol. 18, 1495-1507 (1971).

Pfeiffer, S. E., E. H. Herschman, J. Lightbody, L. Levine and G. Sato. Cell Surface Antigenic Changes as a Function of Culture Conditions. J. Cell Physiol. Vol. 78, 145-152 (1971).

Herschman, H. R., Synthesis and Degradation of a Brain Scientific Protein by Cultured Clonal Human Glial Cells. J. Biol. Chem. 25, pp. 7569-7571 (1971).

Herschman, H. R., S-100 Protein Accumulation in Developing Animals and Cell Cultures. In "Cellular Aspects of Neural Growth and Differentiation: (Ed. D. C. Pease) UCLA Forum in Medical Sciences, University of California Press p. 491-498 (1971).

Costin, A., D. Hafeman, T. Tarby, C. Cotman and H. R. Herschman, Electrophysiological Correlates of Structural Modifications of Brain Tissue by Enzymes and by Antigen-Antibody Reactions to CNS Particulate Functions. Int. Cong. of Physiology, Munich, 1971. (Abstract).

Herschman, H. R., Synthesis and Degradation of an Organ-Specific Protein by Cultured Glial Cells. Int. Society for Neurochemistry, Budapest, p. 26 (1971) (Abstract)

Dravid, A. R., R. E. Wimer and H. R. Herschman, Biochemical Basis for genetically determined brain weight phonotypes. American Soc. of Neurochemistry, Seattle, Wash. March, 1972. (Abstract).

Herschman, H. R., J. Breeding, and J. Nedreud, Sialic Acid Masked Cryptic Cell Surface Antigens on Cultured Cells. J. Cell Physiol. (In Press)

Herschman, H. R., Cell Surface Changes as a Result of Viral Transformation in Membrane. Science (Ed. C. Fred Fox) Sinaurer Assoc. (In Press).

Herschman, H. R., and C. Cotman, Serological Specificity of Brain Sub-cellular Organelles. I. Antisera to Rat Brain Synaptosomes. J. Imm. (In Press).

Costin, A., C. Cotman, D. R. Hafemann and H. R. Herschman, Effect of antibrain synaptosomal fraction serum and complement on evoked potentials and impedance. Experientia (In Press).

12. SCOPE OF THE PROJECT

This project is concerned with the development and 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 brain.

We have embarked upon a program, using serological and radioisotopic tracer methods to study the control of the synthesis and degradation of brain specific proteins in both clonal cell populations and developing neural tissue. By comparing the controlling factors on the synthesis and degradation of organ-specific proteins 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 nerve ending particles, or synaptosomes. 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.

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.

Many other workers in a variety of universities and research laboratories are concerned with the specific subject of functional cell cultures of the nervous system and synaptosomal structure. Our laboratory maintains a constant communication with a number of groups active in these areas.

14. TECHNICAL PROGRESS IN FY 1972

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, Lighbody, 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.

There are contradictory reports in the literature concerning both (a) the types of neural cells which contain this protein, and (b) its synthesis and degradation in brain. One of our objectives in isolating cell lines that continue to synthesize this protein was to establish an experimental system in which these questions could be examined without the presence of complicating issues, including heterogeneous cell populations and the existence of a blood-brain barrier. In order to devise a specific and quantitative assay for incorporation of radioactive amino acids into S100, I chose to work with the CHB_4 cell line, since it accumulates this protein at all times. A technique for specifically and quantitatively isolating radioactively labeled S100, a brain-specific protein, from cultured human glial cells (CHB_4) has been developed. The necessary specificity is achieved by precipitation of labeled S100 in antibody excess with carrier S100 and anti-S100 antibody, followed by acrylamide gel electrophoresis of the solubilized precipitate in the presence of sodium dodecyl sulfate. The specificity of the method is shown by applying the technique to cell lines which do not synthesize S100. Quantitative recovery is demonstrated by a second precipitation from a labeled glial cell extract. The differential rate of S100 protein synthesis in confluent CHB_4 cells is 0.06%. The S100 protein in confluent cultures of CHB_4 appears to be quite stable when compared to total protein, and is not rapidly degraded.

Recent experiments with the rat astrocytoma cell cultures suggest that the S100 protein is not synthesized while these cells are in exponential phase. These results suggest the exciting possibility that the initiation of S-100 protein synthesis in this cell line is a density-dependent contact mediated induction of the synthesis of an organ-specific protein.

2. Synthesis of 14-3-2 in Cultured Neuroblastoma Cells.
Moore and his colleagues have isolated a protein, termed 14-3-2, which has been shown by serological techniques to be specific for neural tissue, and to be present primarily in neurons. We have, during the past year, made antisera to this protein, and confirmed its neural specificity. We have analyzed the major clonal isolates of the C1300 mouse neuroblastoma (Clone NB41 of Sato, Clone C1a of Schubert, Clones N18 and N4 of Niremberg) and found them all to be negative. Recently, however, we have received a human neuroblastoma (IMR32) which appears to accumulate this antigen in culture. Our work with this population of cells will be similar in scope and nature to that described for S-100 in section 14.1.

3. Biochemical basis for genetically determined brain weight phenotypes.
Recent studies have demonstrated a striking amount of variability among genetically distinct stocks of house mice in both absolute and relative amounts of brain tissue and in several aspects of brain chemistry. At present, brains of mice of different stocks (inbred strains or selected lines) are known to vary in their size and weight. It is not known whether these genetically determined variations in brain weight (and size) originate in variations in cell size or cell number, nor is it known whether neurones and glial cells are equally involved. To ascertain the involvement of neurones and glial cells, we have measured two nervous system specific proteins 14-3-2 and S-100 as potential markers

for neurons and glial cells. Studies on DNA measurements on these brains indicate that variations in brain weight originate primarily in cell number. Existence of a secondary variation is indicated by the finding that DNA per unit weight in high brain weight mice was significantly lower as compared with controls. No significant difference was observed in DNA per unit weight between controls and low brain weight mice although the values were somewhat higher in the low brain weight mice. Studies on the cell specific proteins 14-3-2 and S-100 indicate that the neuronal protein, 14-3-2 remains constant per unit weight in all three groups of mice whereas the glial cell (astrocytes) specific protein, S-100 is significantly decreased as compared with the controls. The decrease was much more pronounced in the low brain weight mice. These studies suggest that genetically determined variation in brain weight is a consequence of of variation in cell number and the cells involved in the variation in number may be largely glial cells.

4. Antisera to Nerve endings.
In collaboration with Dr. Carl Cotman of U.C. Irvine. 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 organelles 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 resistence to enzymic digestion of the antigens have been characterized. Cross reaction of a variety of vertebrate nerve ending particle preparations have been quantitated.

This antisera has been used in electrophysiological experiments designed to test its effect in EEG, flash evoked potential, and impedance (in collaboration with Drs. Anatole Costin and Dennis Hafemann). These results while somewhat complicated and difficult to summarize suggest that antisera are able to promote specific physiological effects.

Recently we have isolated the 7S gamma globulin of this antisera and labelled a portion with I^{125}. Thirty percent of the TCA precipitable counts are bound by saturating concentrations of synaptosomes, while little or no uptake of labelled antibody is observed with comparable amounts of liver membrane.

15. EXPECTED RESULTS IN FY 1973

1. We hope to be able to clarify the nature of the synthesis and and degradation of S-100 and 14-3-2 in clonal glial and neuroblastoma cells respectively. 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.

2. Using inhibition of binding, we plan to solubilize, isolate and characterize the synaptosome membrane antigens. We plan to compare these antigens with the acetycholine receptor by using labelled α-Bungarotoxin. We will also prepare ferritin-labelled antisera and localize the antigens with the aid of the electron microscope. Developmental studies are also planned.

3. Studies of genetically determined brain weight phenotypes will be extended to specific brain regions and to developing animals.

16. EXPECTED RESULTS IN FY 1974

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, 4) the induction and isolation of neural tumors, 5) continued investigations of the roles of brain-specific soluble proteins and 6) the factors affecting protein synthesis in developing brain.

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	2	2	3
(b) Other Tech.	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$
TOTAL:	2 $\frac{1}{2}$	2 $\frac{1}{2}$	3 $\frac{1}{2}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 35,700	$ 36,000	$ 49,000
(b) Materials and Services	11,900	13,400	18,100
Sub-Total Direct Project Support	$ 47,600	$ 49,400	$ 67,100
(c) Indirect Expenses *	23,700	21,200	25,200
TOTALS:	$ 71,300	$ 70,600	$ 92,300

* 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 1972

Gerschenson, L. E., Myrna Andersson, Regulation of the Pyruvate kinase of an Established Rat Liver Cell Line (RLC) by Insulin, Serum and Glucose, Biochem. Biophys. Res. Comm. 43 (1971) 1211-1218.

Okigaki, T. and L. E. Gerschenson, Chromosomal Rosettes in an Air-Dried Preparation Made without Colchicine, Chromosome Information Service, 11, 12-13 (1970).

12. SCOPE OF THE PROJECT

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

A cell line (RLC) derived from adult rat liver, has been established in our laboratory and adapted to grow in a chemically defined medium. Several liver-parenchyma, organ-specific morphological and functional characteristics have been found in this cell line.

The regulation of the enzymes pyruvate kinase and tyrosine α-ketoglutarate transaminase by the hormones insulin and dexamethasone have been studied as well as the control of general protein and RNA synthesis.

Two problems, intimately related are now under study: 1) the mechanism of hormonal regulation of RNA and protein synthesis in RLC cells. 2) The isolation of hormonal receptors in the same cells.

13. RELATIONSHIP TO OTHER PROJECTS

There is a strong relationship and collaboration between this project and others throughout the world: in this Laboratory, I. Harary, J. de Vellis, H. Herschman and J. F. Mead, at UCLA; M. Davidson, in the USA: B. Thompson (NIH), J. de Paolo (NIH), G. Tomkins (UCSF), F. T. Kenney (Oak Ridge), V. R. Potter (Univ. Wisconsin), A. Moscona (Univ. Chicago), H. Eagle and A. Schwartz (A. Einstein School of Med.), B. Ephrussi (France), H. Kruger (Berlin), P. Malpoix (Belgium) etc.

14. TECHNICAL PROGRESS IN FY 1972

The enzyme pyruvate kinase in RLC cells appears to be under the influence of hormonal and nutritional regulatory mechanisms similar to those modulating the enzyme in whole rat liver. The insulin effect, increasing the pyruvate kinase activity, appears to be dependent upon de novo RNA synthesis, while the stimulation of the same enzyme activity by glucose appears to be totally independent of protein synthesis.

The addition of glucose or lactic acid to the culture medium inhibited the insulin effect, suggesting a regulatory mechanism for pyruvic kinase in which insulin would be an inducer and either lactic or pyruvic and repressors of the insulin effect.

The synthetic corticosteroid dexamethasone was found to be an inducer of the enzyme tyrosine α-ketoglutarate transaminase inducer of the enzyme tyrosine α-ketoglutarate transaminase in RLC cells. Insulin alone was found to be not effective in eliciting an increase of the same enzyme. However, when added after 18 hours of previous incubation with dexamethasone (the steroid

effect is then maximal) induces a 2 to 3-fold increase which is not inhibited by actinomycin D, while the addition of cycloheximide inhibited it. This insulin effect was observed with concentrations as low as 1μU of insulin per ml of medium. The addition of similar concentrations of insulin derivatives or precursors did not have any effect. The effect of insulin was found to be present even in the absence of amino acids and/or glucose.

Insulin was found to increase 20-30% the general rate of protein synthesis without preincubation with dexamethasone, and this last hormone increases 10-20% the rate of RNA synthesis. From studies with protein and RNA synthesis inhibitors, it appears that the insulin effect is elicited at the translational level, while the dexamethasone effect takes place at the transcriptional level.

15. EXPECTED RESULTS IN FY 1973

During this year it is expected:

1) To advance our knowledge on the mechanism by which Insulin and Dexamethasone regulate the genetic expression of RLC cells. A cell-free system will be established to study this topic.
2) To partially isolate the receptors for both hormones.
3) To study the effect of ionizing radiation on the hormonal effects.

16. EXPECTED RESULTS IN FY 1974

1) We will continue to study the hormonal regulation of protein synthesis, stressing probably the role of initiation and termination factors in the case of Insulin.
2) A further purificaton of hormonal receptors is expected.

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 - 1972

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

7. Person in Charge:	8. Project Term:
Jean S. de Vellis	From: 1964 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2 ½	2 ½	3
(b) Other Tech.	½	½	1
TOTAL:	3	3	4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 35,600	$ 38,000	$ 44,000
(b) Materials and Services	12,700	13,500	17,100
Sub-Total Direct Project Support	$ 48,300	$ 51,500	$ 61,100
(c) Indirect Expenses *	23,700	21,800	22,700
TOTALS:	$ 72,000	$ 73,300	$ 83,800

* 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 1972

de Vellis, J., D. Inglish and G. Augusti-Tocco: The Influence of Hormones on Neurite Formation in a Neuroblastoma Cell Line, p. 191 Third Intern. of Soc. Neurochemistry, Budapest, Hungary, July, 1971. (Abstract).

Shinwari, M. A. and J. De Vellis: Effects of Cortisol (C) and Norepinephrine (NE) on the uptake of Analogs of Amino Acids and Glucose into cultured Glial cells. Third Meet. Amer. Soc. Neurochem. Seattle, March 20-23, 1972. (Abstract).

de Vellis, J. and G. Brooker: Effect of Catecholamines on Cultured Glial cells: Correlation Between Cyclic AMP Levels and Lactic Dehydrogenase Induction. Federation Proceedings, April 1972. (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, Absel pp 25-39 (1971).

de Vellis, J. and D. Inglish: Hormonal Induction of Enzymes in culture Glial Cells: Differential inhibition by α-Amanitin, Cordycepin and Actinomycin D. 23rd. Ann. Meet. Tissue Culture Assoc. 1972. (Abstract).

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 chracterize 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, L. E. Gerschenson and J. Byfield 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 1972

1. Hormonal Induction of Enzymes in Cultured Glial Cells: Differential Inhibition by α-Amanitin, Cordycepin and Actinomycin D. The rat brain astrocytoma cell line, RGC_6, is characterized by several specialized functions of glial cells, including the cortisol induction of glycerol phosphate dehydrogenase (EC 1.1.1.8) (GPDH) and the induction of lactate dehydrogenase (LDH) by norepinephrine (NE). Cyclic AMP appears to be the mediator for the action of NE but not for cortisol (J. Neurochem. 15, 1061, 1968; Cellular Aspects of Neural Growth and Differentiation, D. Pease, Ed., pp 23-32, Univ. Calif. Press, 1971; de Vellis and Brooker, see below). In the present study, the effect of inhibitors of RNA synthesis on GPDH and LDH inductions was investigated. Cells were grown for 9 days to a density-inhibited confluent monolayer, using Ham's F_{10} medium supplemented with 10% fetal calf serum. Alpha-Amanitin (10^{-5}M), a specific inhibitor of RNA polymerase (RP'ase) II (Science, 170, 447, 1970), blocked the induction of GPDH but not LDH. Actinomycin D (AMD), which at 0.05 μg/ml inhibits only rRNA synthesis, blocked the induction of GPDH but not LDH. AMD which at 1 μg/ml inhibits all RNA syntehsis, and cordycepin, an inhibitor of mRNA (PNAS 67, 1878, 1970), blocked GPDH and LDH inductions. The data suggest that both enzyme inductions require de novo synthesis of mRNA but that their mRNAs are transcribed by different nucleoplasmic RP'ases. The GPDH mRNA is synthesized by RP'ase II and therefore the LDH mRNA is probably produced by RP'ase III. The effect of low level of AMD suggests that the synthesis of GPDH mRNA is as sensitive as rRNA to AMD.

2. Effect of Catecholamines on Cultured Glial Cells: Correlation between Cyclic AMP Levels and Lactic Dehydrogenase Induction (Jean de Vellis and Gary Brooker). The regulation of cyclic AMP level and its relationship to LDH induction was investigated in the rat glial cell line, RGC_6. Dibutyryl cyclic AMP (0.5 mM) induced LDH but not GPDH. Cortisol did not increase cyclic AMP or LDH. Following the addition of catecholamines (10^{-9} to 10^{-5}M) there was a rapid rise in cyclic AMP (up to 30 fold) which preceded LDH induction. However, only a 6 fold increase in cyclic AMP was necessary for maximal LDH induction. Submaximal concentrations of catecholamines had a correlative effect on cyclic AMP and LDH levels. Isoproterenol, NE, epinephrine, epinine, octopamine and phenylephrine were active and blocked by propranolol. Tyramine, histamine and dopamine were inactive. The first 3 hours after NE represented a cyclic AMP dependent RNA phase, whereas the subsequent LDH synthesis (after 6 hours) did not require elevated cyclic AMP levels. The effect of catecholamines

on glial cells appears mediated by a beta receptor and the rise in LDH is initially triggered by a rise in intracellular cyclic AMP.

3. Effects of Cortisol (C) and Norepinephrine (NE) on the Uptake of Analogs of Amino Acids and Glucose into Cultured Glial Cells. (M. A. Shinwari and J. de Vellis). The rat brain glial cell line, RGC6, is a target cell for cortisol and norepinephrine (see above). Cyclic AMP is the intracellular mediator for the action of NE but not for C. The incorporation of appropriate labelled precursors into lipids, proteins RNA and DNA was altered by treatment with C or NE. In order to assess the metabolic significance of these changes it was deemed necessary to measure the effects of C and NE on membrane permeability. C6 cells were grown for 8 days to a density-inhibited confluent monolayer, using Ham's F10 medium supplemented with 10% fetal calf serum. Time course of uptake and concentration curves were carried out for each compound tested. Incubation with radioisotopes lasted for 20 min. in Krebs Ringer Phosphate buffer or culture medium. Treatment with C for 5 days progressively decreased the uptake of ^{14}C-aminoisobutyric acid (AIB) and ^{14}C-2-deoxy-glucose (dG) to a final level of 60% of control. The time course was a mirror image of GPDH induction which after an 8-fold rise reaches a new steady state in 5 days. One hour following the addition of NE to cell cultures the uptake of AIB and cycloleucine was increased to 130% of control level whereas dG was decreased to 65% of control. Dibutyryl cyclic AMP had similar effects. At 24 h the stimulatory effect of NE on AIB and cycloleucine uptake had disappeared. These effects of NE correlated well with the rise and fall in the intracellular level of cyclic AMP. The data suggest that cyclic AMP is the mediator of NE effect on membrane permeability. The data also show that the effect of C and NE on membrane permeability of glial cells is differential and time-dependent.

15. EXPECTED RESULTS IN FY 1973

A major effort of this program will be directed to the further characterization of the mechanism of action of cortisol and norepinephrine on several processes of their target cell, the brain astrocytes. The rat glial cell line, RGC6, which has retained several of the putative functions of glial cells (S-100 protein, inductions of glycerol phosphate dehydrogenase and lactate dehydrogenase, catecholamine sensitive adenylcyclase) will continue to be the preferred system of investigation. A comparative study of cortisol and norepinephrine effects is particularly interesting because the latter but not the former is mediated via cyclic AMP. In collaboration with several postdoctoral fellows and graduate students the following projects will be carried out:

a) Role of nuclear proteins (acidic proteins and histones) in hormonal regulation of enzyme and protein synthesis in glial and neuronal cells in culture (with Dr. Grace Yu). The synthesis and turnover of various nuclear proteins will be investigated by polyacrylamide gel electrophoresis. Cells will be incubated with appropriate labelled precursors at very high specific radioactivity. It is hoped that such experiments will reveal nuclear proteins involved in activation of the genome by hormones, hence suggesting a mechanism for specificity.

b) Influence of cortisol on the rate of synthesis and degradation of brain glycerol phosphate dehydrogenase (with Dr. J. F. Mc Ginnis).

The enzyme, glycerol phosphate dehydrogenase has been purified from the rat brain. Unlike the muscle and liver GPDH enzymes which have been isolated from rats, the brain enzyme is relatively labile. Thus, in addition to establishing the conditions required to purify the enzyme, lability necessitated the development of procedures which promoted stability. Using the purified enzyme as an antigen, it will probably be possible to obtain specific antibodies which can be used to measure the rates of synthesis and degradation of GPDH in a number of situations, such as the induction by cortisol in vivo and in glial cultures, the radiation-induced inhibition of GPDH in the developing rat brain, etc.

Since GPDH induction by cortisol is brain-specific, it seems logical to expect that it plays an important part in brain metabolism. To investigate this aspect, the kinetic properties of the enzyme are being determined with the anticipation that knowledge of these parameters will lead to a better understanding of the role that this enzyme plays in brain tissue.

c) The role of RNA metabolism in the hormonal effects of cortisol and norepinephrine.

In previous experiments it was shown that inhibitors of RNA synthesis block the cortisol induction of GPDH and the norepinephrine induction of LDH. Furthermore recent experiments (see section 14) strongly suggest that the messenger RNAs for LDH and GPDH are produced by different RNA polymerases (isoenzymes). The fact that a low level of actinomycin D blocked only GPDH induction but not LDH induction suggests that de novo ribosomal RNA synthesis is required for GPDH induction. The data suggest three areas of investigations. One project will be to test the effect of hormones on the activity of RNA polymerase isoenzymes. The second area would be to measure the template activity of the chromatin of induced cells and determine the types of mRNAs produced by DNA-RNA hybridization experiments. The third project will involve the analysis of RNA patterns by polyacrylamide gel electrophoresis.

d) The effect of hormones on the permeability of glial cells (with Dr. Shinwari). This project is a continuation of a work reported in section 14. One of the aims is to elucidate the metabolic significance of GPDH and LDH inductions. Furthermore, because norepinephrine is a neurotransmitter substance it may provide a mechanism for the neurons to couple glial metabolism to their functions by activating adenylcylase. It is, therefore, important to find out why LDH is induced by cyclic AMP generated by the action of norepinephrine on glial cells. It is also already apparent that cyclic AMP has other metabolic functions in glial cells.

e) Receptor sites for glucocorticoid hormones in glial and neuronal cell lines.

In collaboration with Dr. B. Mc Ewen, from Rockefeller University, we will study the binding sites for glucocorticoids. In preliminary experiments it was found that rat glial cells retain corticosterone specifically in their nucleus. Furthermore corticosterone is retained preferentially when compared to cortisol. This finding confirms the in vivo observations of Mc Ewen with the rat brain. The hormone receptors of the cytosol and nucleus will be characterized and studied in relation to the induction of enzymes by glucocorticoids.

f) Regulation of cyclic AMP level in glial cells.

In collaboration with Dr. Gary Brooker, International Chemical and Nuclear Corporation will continue the work reported in section 14. Recent experiments indicate that a rapidly turning over protein is involved in the regulation of the level of intracellular cyclic AMP. This aspect will be studied first since it is potentially of fundamental importance.

16. EXPECTED RESULTS IN FY 1974

The projects listed in section 15 will be continued and expanded into areas judged most important by the results of future experiments. In addition, investigations will be undertaken on: (1) histogenesis and functional differentiation in reaggregating cultures of mouse cerebellum, (2) synaptogenesis in neuronal cell cultures, (3) binding sites and specificity of action of cyclic AMP.

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	3 ½	3	4
(b) Other Tech.	½	½	½
TOTAL:	4	3 ½	4 ½

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 49,000	$ 45,000	$ 62,000
(b) Materials and Services	25,100	22,500	28,700
Sub-Total Direct Project Support	$ 74,100	$ 67,500	$ 90,700
(c) Indirect Expenses *	31,600	26,100	31,900
TOTALS:	$ 105,700	$ 93,600	$ 122,600

* 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 1972

Hüttermann, Jürgen, John 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. Chem. 3, 117-129 (1971).

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. 54, (7), 2899-2909 (1971).

Theard, L. M., F. C. Peterson, and L. S. Myers, Jr.: Nanosecond Pulse Radiolysis Studies of Aqueous Thymine Solutions. J. Phys. Chem. 75, 3815-3821 (1971).

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. Proc. of Biophysical Society Annual Meeting (Feb., 1971), New Orleans, La., p. 178a, WPM-I-11 (Abstract).

Myers, L. S., Jr.: Formation and Reactions of Free Radicals Produced by Pulse Radiolysis of Nucleic Acids and Nucleic Acid Constituents. Radiation Research 47, 255-256 (1971) (Abstract).

Myers, L. S., Jr.: Macromolecules of Biological Interest: I. Nucleic Acids, II. Proteins, and III. Polysaccharides. Three chapters in "Radiation Chemistry of Biological Molecules" M. Dole, ed. (in press).

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; D. A. Vroom, 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. 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 1972

Radiation Chemistry of Macromolecules of Biological Interest: I. Nucleic Acids, II. Proteins, and III. Polysaccharides: Three chapters have been prepared for inclusion in a book titled "Radiation Chemistry of Macromolecules" (M. Dole, ed.) which is scheduled to be published in the calendar year 1972. The chapters on nucleic acids and proteins include detailed discussions of radiation-induced free radical formation and chemical changes in solid constituents, low molecular weight fragments, and the macromolecules themselves. In addition, recent developments on reactions of these substances with hydroxyl free radicals and hydrated electrons produced by radiolysis of water are reviewed with emphasis on pulse radiolysis results. The chapter on polysaccharides discusses several specialized topics.

Nanosecond Pulse Radiolysis Studies of Aqueous Thymine Solutions: Nanosecond pulse radiolysis of aqueous thymine solutions has been studied by producing optically detectable concentrations of transients with 10-nsec electron pulses from a Linac and employing an optical system with a signal rise time of 5 nsec. In deaerated 10^{-2} M thymine solutions containing 1 M ethanol at pH 6.0, thymine radical anion, T^- (λ_{max} 325 nm), is present at the end of the pulse and its decay over the subsequent 10^{-6} sec is only slight. At pH 4.0, T^- decays exponentially with a half-life of ca. 10^{-7} sec, and upon decreasing the pH to 3.5 the decay half-life decreases in proportion to the increase of acid concentration, suggesting that T^- decays via $T^- + H_3O_{aq}^+ \rightarrow TH + H_2O$ with a reaction rate constant of $(6.4 \pm 0.3) \times 10^{10}$ $M^{-1}sec^{-1}$. The slow decay of T^- at pH 6.0 suggests that for the reaction $T^- + H_2O \rightarrow TH + OH^-$, $k \leq 10^4$ M^{-1} sec^{-1}. An absorption signal presumed attributable to TH is present following decay of the T^- signal, with λ_{max} also at 325 nm and an intensity about half that of T^-. Formation of the H-atom adduct of thymine, TH', produced via the reaction of $H + T \rightarrow TH'$ was observed to occur during the period 10^{-8} to 10^{-6} sec following the pulse for deaerated 10^{-2} M thymine at pH 0.65. The rate constant for TH' formation is $(6.8 \pm 0.2) \times 10^8$ $M^{-1}sec^{-1}$, and the absorption spectrum (λ_{max} 400 nm) of TH' is different from that of TH. The data suggest that H-atom addition to thymine occurs at the 5,6 double bond while protonation of T^- occurs elsewhere. The submicrosecond decay of T^- and TOH

resultant from their reactions with oxygen was observed; rate constants for the reactions are 4.3×10^9 and 1.9×10^9 $M^{-1}sec^{-1}$, respectively. (In cooperation with Dr. L. M. Theard, Gulf Radiation Technology, San Diego, California.)

Electron Spin Resonance Studies of Free Radicals in Irradiated Single Crystals of 5-Methylcytosine: This study has been finished with the following results:

Exposure of single crystals of 5-methylcytosine to ionizing radiation gives rise to electron spin resonance (E.S.R.) spectra which at room temperature can be explained as resulting predominantly from two chemically distinct stable free radicals produced in the crystals. One is formed by addition of a hydrogen atom to the 6-position of the pyrimidine ring. Its essentially isotropic hyperfine spectrum is generated by interaction of the unpaired electron in a 2p-orbital of carbon atom $C_{(5)}$ with three equivalent protons of the 5-methyl group and two non-equivalent protons attached to carbon $C_{(6)}$, all protons being in the β-position. The second radical results from removal of a hydrogen atom from the 5-methyl group. The unpaired electron of this radical is localized mainly in 2p-orbitals of the methyl carbon and of ring carbon $C_{(6)}$ giving rise to a hyperfine spectrum from interaction with the two remaining α-protons of the methyl group and the α-proton attached to $C_{(6)}$. All couplings are anisotropic with principal values of $A_x = 24.9$, $A_y = 16.3$ and $A_z = 8.6$ gauss for the methyl protons and $B_u = 12.5$, $B_v = 8.6$ and $B_w = 2.1$ gauss for the $C_{(6)}$ proton. Measurements at low temperatures reveal the presence of a third type of radical featuring a doublet ESR resonance which is tentatively assigned to the radical anion of 5-methylcytosine. These findings are discussed in connection with results reported for related compounds. (In collaboration with Drs. J. Hüttermann (Postdoctoral Scholar) and J.F. Ward (Sub-Cellular Radiobiology Section)).

Pulse Radiolysis of DNA: Studies of the site of attack by hydroxyl free radical (OH) on DNA have been continued with an intensive effort to determine relative extinction coefficients of radicals formed on the different bases and nucleotides. This information will permit synthesis of an improved spectrum based on nucleotide absorptions for comparison with observed DNA spectra. In addition decay of DNA radicals formed by OH attack has now been measured. Past results obtained on a microsecond time scale have shown only that decay is slow. Techniques have been developed for following decay over a 200 millisecond period. The optical absorption due to DNA radicals decays after a pulse of a few thousand rads by a first order process with a half-life of about 50 milliseconds. This is a surprising result. It was expected that decay would be second order, indicating a radical-radical recombination process. Reactions responsible for the decay have not been identified. Studies of reactions of DNA with hydrated electrons also have been carried out. These are in a preliminary stage.

Transfer of Electrons from Thymine Anion Radical to 5-Bromouracil: It has been suggested that one mechanism by which substitution of 5-bromouracil (BU) for thymine (T) in DNA causes sensitization to radiation involves transfer of electrons from other bases to BU (Zimbrick, Ward, and Myers, Int. J. Rad. Biol. 16, 505 (1969)). We have observed this reaction in a model system by pulse radiolysis. Radiolysis of thymine in dilute aqueous argon saturated solutions containing 1 M t-butanol produces hydrated electrons, e^-_{aq}, which react with thymine to give thymine anion radical ($T^{\cdot}$). This substance has an optical absorption maximum at

$\sim$ 325 nm and with doses of 1000 rads, a half-life of $\sim$ 60 μsec. Radiolysis of BU under similar conditions produces uracil which does not abosrb at 325 nm. (Hydrated electrons react by dissociative electron attachment with BU to give uracilyl radical which immediately abstracts hydrogen from the butanol to give uracil.) Radiolysis of solutions containing mixtures of T and BU in proportions such that e^-_{aq} reacts mainly with T, gives an absorption with λ_{max} 325 whose intensity is consistent with competition for e^-_{aq} by T and BU, and whose half-life decreases with increasing BU concentration. The decrease in half-life is believed to be due to transfer of electrons from $T^{\cdot -}$ to BU. The rate constant for the reaction is of the order of 10^9 $M^{-1}sec^{-1}$. Similar experiments under conditions such that ·OH instead of e^-_{aq} attacks T and BU do not show a similar dependence of decay rate on BU concentration. (In collaboration with L. M. Theard, Gulf Radiation Technology, San Diego, Calif.)

Pulse Radiolysis Study of the Thymine Anion Radical: Previous ESR studies of the reaction e^-_m + T (mobile electrons plus thymine) in alkaline ices at low temperatures have suggested that the product has H· added to $C_{(5)}$ (TH!). Pulse radiolysis studies in acid solution have indicated that e^-_{aq} + T followed by $T^{\cdot -} + H^+$ gives a product different from TH!, TH·. Reactions of T with e^-_{aq} have now been investigated by pulse radiolysis over the pH range 4 to $\sim$ 15 (4N NaOH). In particular a tautomeric change from TH· to TH!, or a different reaction path in alkaline solutions has been searched for. Results have been compared with results of the reaction DHT + ·OH (and ·H) in alkaline solutions which gives TH!. Comparison of spectra show that TH! is not formed from T + e^-_{aq} during the period of observation ($\sim$ 1 msec) at any pH. The effect of ionic state of T on spectra and decay of radicals was also investigated. (In collaboration with L. M. Theard, Gulf Radiation Technology, San Diego, Calif., and R. F. Holroyd, Brookhaven National Laboratory.)

Other Studies: Attempts are in progress to study by pulse radiolysis reactions in systems containing thymine, deoxyribose, or cytosine plus cysteine. Reactions initiated by both OH and hydrated electrons are being investigated. Initial pulse radiolysis studies have also been begun on the dissociative reactions caused by hydrated electrons when they interact with 5-halouracils or thymine dimer. Investigation by ESR of irradiated ices and of a photoradiolysis phenomenon observed on pulse radiolysis of thymine in the presence of ultraviolet light have been continued. (The pulse radiolysis studies were in collaboration with L. M. Theard, Gulf Radiation Technology, San Diego.)

15. EXPECTED RESULTS IN FY 1973

Investigations of the radiolysis of nucleic acids will be continued and extended to nucleoproteins. General goals will be to increase our understanding of radiation effects and to develop means of modifying these effects. Pulse radiolysis, ESR spectroscopy, and other more conventional radiation chemistry techniques will be used to study mechanisms of action of radiation, both direct and indirect, on nucleic acid constituents, nucleic acids, nucleoproteins, and radiation modifiers. Pulse radiolysis experiments will be conducted for the most part in collaboration with D. A. Vroom, Gulf Radiation Technology, San Diego, Calif. Specific experiments follow:

Pulse Radiolysis Investigation of a Proposed Mechanism by which 5-Bromouracil Sensitizes DNA to Radiation: It has been proposed that reactions by which 5-bromouracil (BU) sensitizes DNA to radiation include transfer of electrons

from other bases to 5-bromouracil, cleavage of 5-bromouracil to give uracilyl radical and bromide ion, and reaction of uracilyl radical with a deoxypentose moiety to cause a strand break and formation of the foreign base, uracil. Every step of this mechanism has been verified with model compounds (bases and nucleotides) by either this Section or the Sub-Cellular Radiobiology Section. We plan to grow E. Coli DNA in which nearly all of the thymine has been replaced by BU, using a bacterial strain obtained from Roger Hewitt at the M. D. Anderson Hospital in Houston, Texas and to observe its reaction with hydrated electrons by pulse radiolysis. Normal (thymine containing) DNA reacts with hydrated electrons to give a product which has an optical absorption maximum at about 320 nm and decays very slowly. The BU containing DNA, if the hypothesis is correct, should give either a much more rapidly decaying product, or one which has a much lower extinction coefficient.

Mechanisms of Action of Sulfhydryl Compounds and Other Radiation Modifiers: We have begun, and plan to continue pulse radiolysis studies of complex systems in which it is possible to observe 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 living cells and are very likely an important source of biological damage. The action of radiation modifiers such as sulfhydryl compounds, N-ethylmaleimide, and oxygen also may involve such reactions. Reactions initiated by both hydroxyl free radicals (OH) and hydrated electrons (e^-_{aq}) will be investigated. For experiments with OH generated radicals, N_2O saturated solutions containing a nucleic acid (or constituent) will be exposed to a radiation pulse, and absorption spectra and rates of decay of radicals will be observed. Concentrations can be varied so that OH reacts predominantly with one or another of the components or equally with the two, making it possible to observe reactions a, b, or c. Nucleic acid constituents to be investigated initially are thymine, cytosine, deoxyribose, and the saturated analog of thymine, dihydrothymine. Similar experiments will be done with radicals generated by e^-_{aq} in argon saturated solutions containing an OH scavenger such as t-butanol.

Transfer of Electrons from Nucleic Acid Constituents to 5-Bromouracil: This reaction has been demonstrated for transfer from thymine anion radical ($T^{\cdot-}$) and cytosine anion radical, and a rate constant has been determined by pulse radiolysis for the $T^{\cdot-}$ reaction. However, the degree to which electron transfer is a general reaction of nucleic acid constituents has not been determined. We plan to study the possibility of electron transfer to BU from anion radicals of adenine, and thymidylic, uridylic, cytidylic, adenylic, and guanylic acids, and to determine the rate constants in those cases where transfer occurs. An investigation will also be made to determine whether normal DNA which has reacted with e^-_{aq} will transfer electrons to BU in solution.

Pulse Radiolysis Study of Photo-Radiolysis Phenomena: Study of the combined effects of photolysis and pulse radiolysis will be continued. Emphasis will be placed on attempts to identify photoradiolysis products in pure water and in aqueous solution and to determine the mechanisms by which they are formed. A detailed study of photoradiolysis effects in pure water is expected to be particularly informative, because the results may be interpretable in terms of effects of photolysis of the primary species of water radiolysis.

Other Pulse Radiolysis Studies: Reactions of hydrated electrons (e^-_{aq}) with nucleic acids will be investigated in greater detail than heretofore: optical absorption spectra of nucleic acid radicals formed by reactions of e^-_{aq} and decay rates will be determined; attempts will be made to determine the rates of the extremely rapid dissociation processes of 5-halouracils and the thymine dimer following electron capture; rates of protonation of anion radicals will be measured.

ESR Studies of the Radiolysis of Halouracils in the Solid State and in Ices: These studies are related to the pulse radiolysis work with the same compounds and are an attempt to gain further information about reactions of halouracils, especially 5-bromouracil, with electrons, and the subsequent secondary reactions. The pure compounds and compounds dissolved in neutral, alkaline, and acid glassy ices will be irradiated, and ESR spectra will be determined and analyzed to gain information about intermediate species.

ESR of Irradiated Nucleic Acids: At least seven different spectra have been reported for irradiated nucleic acids. Conditions leading to the different spectra have not been identified. Experiments will be done in an attempt to determine the influence of conditions on the structures of radicals formed. Variables to be considered are: protein contamination, secondary nucleic acid structure, temperature, moisture content, oxygen content, and metal ion content.

Other projects: Several exploratory projects will be carried out. These will include experiments with particles generated by the UCLA Biomedical Cyclotron: 22 MeV protons, 11 MeV deuterons, and energetic He^{3+} and He^{4+} ions. Samples (nucleic acids, etc) will be exposed and ESR spectroscopy and other techniques will be used to determine the effects of these particles and to compare them with effects of γ-rays and 10 MeV electrons. Also, attempts to determine the mechanism of splitting of pyrimidine dimers by ionizing radiation will be continued.

16. EXPECTED RESULTS IN FY 1974

Research will be continued with the same general goals, but with continued attempts to extend studies into more complex systems to facilitate application of results to living organisms. Pulse radiolysis and ESR spectroscopy will be almost certain to continue to provide important and otherwise almost impossible to obtain information. Attempts to find ways to modify radiation effects by altering early radiation induced chemical reactions are expected to continue. Examination of the role of hydrated electrons in radiation damage will be continued, probably with studies of electron reactions with nucleic acid in solution and in the solid state, of electron transfer reactions, and of secondary reactions of anion radicals. If preliminary experiments with cyclotron produced particles indicate that further work in this area will be profitable, considerable emphasis will be placed on such studies. It is hoped that our studies will advance to the point where the role of protein in radiation damage of nucleic acids can be investigated.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: SUB-CELLULAR RADIOBIOLOGY

3. AEC Budget Activity No.:	4. Date Prepared:
06 01 02	April - 1972
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 1972	FY 1973	FY 1974
(a) Scientific	2 1/2	2	3
(b) Other Tech.	1/4	3/4	3/4
TOTAL:	2 3/4	2 3/4	3 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 30,500	$ 34,000	$ 46,000
(b) Materials and Services	13,300	13,900	16,100
Sub-Total Direct Project Support	$ 43,800	$ 47,900	$ 62,100
(c) Indirect Expenses *	23,700	20,100	23,500
TOTALS:	$ 67,500	$ 68,000	$ 85,600

* 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 1972

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. 3, 117-129 (1971).

Ward, J. F.: Deoxynucleotides - Models for Studying Mechanisms of Strand Breakage in DNA. 1. Protection by Sulphydryl Compounds. Int. J. Rad. Phys. and Chem. 3, 239-249 (1971).

Ward, J. F. and I. Kuo: Model Systems for Investigations of the Mechanisms of Single Strand Break Production in γ-Irradiated Nucleic Acids. Rad. Res. 47, 291 (1971). (Abstract)

Lewis, H. L. and J. F. Ward: Mechanism of the Hydration Effect of Freeze-dried T_1 Bacteriophage. Rad. Res. 47, 294 (1971). (Abstract)

Ward, J. F. and J. D. Zimbrick: A Molecular Mechanism for 5-Bromouracil Radiosensitization. Biophysical Society Abstracts, SuAM-C_3 (1972). (Abstract)

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 1972 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.

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, K. C. Smith and H. Kaplan, Stanford Univ., Palo Alto, California, J. D. Zimbrick, Univ. of Kansas, Lawrence, Kansas.

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 1972

Mechanisms of strand break production in DNA: We have continued use of deoxynucleotides as model compounds for investigating these processes. Here breakage of the sugar phosphate bond liberating inorganic phosphate is equivalent to the production of a strand break in DNA.

1. Identification of compound formed after release of inorganic phosphate, (iP): Uniformly ^{14}C labelled (U.L.) deoxycytidylic acid was used to determine the number of sugar carbons which remain attached to the base moiety after release of the phosphate group: The specific activity of the starting material was determined (referring ^{14}C d.p.m. to UV absorption). After irradiation, products which had lost iP were separated from starting material by absorption of the latter on to an anion exchange column. The specific activity of the products was determined; any loss of C atoms from the U.L. dCMP would cause reduction in specific activity. Two products were isolated which had specific activities different from the starting material, Product A 40% of the yield (G = 0.13) had a specific activity 0.63 of original and B (G = 0.18) 0.79 of original. Therefore, A has 2 C atoms of the sugar attached and B has 3 C atoms attached to the base. (Original compound had 4 C atoms in base moiety and 5 in the sugar.)

It was necessary in a separate experiment to determine the extinction coefficients (ϵ) of the products. This was achieved using 2 ^{14}C labelled dCMP and the products had ϵ of 10,000, i.e. closer to dCMP than C.

U.L. thymidylic acid (TMP) is not commercially available and a similar experiment is therefore not feasible with this compound. Three products which did not contain phosphate were isolated from irradiated TMP in the yields G = 0.1, 0.06 and 0.2.

2. Malonaldehyde as a radiation product: Other workers have estimated the yield of malonaldehyde (MA) from irradiated nucleic acids and have found values comparable to the yield of strand breaks. Kapp and Smith (Rad. Res. 42, 34 (1970)) obtained $G_{(sb)} = 0.4$ and $G_{(MA)} = 0.24$. We measured MA yields from irradiated solutions of deoxynucleotides under oxygenated conditions and found much lower values. G(MA) from dCMP = 0.05, dGMP = 0.06, dAMP = 0.15, TMP = 0.09 and thymidine 3'5' diphosphate = 0.04. Hence, the involvement of MA formation in the production of an sb is doubtful.

3. Thymidine 3'5' diphosphate (TDP) as a model compound: Since both the 3' and the 5' hydroxyls of the deoxyribose are phosphorylated (as in DNA) this compound could be a better model.

Measurements of iP yields showed that the initial yield is low G = 0.2 in O_2 saturated solution but increased rapidly on standing at pH 7 to G = 0.55. If the sample is cooled to 0°C or adjusted to pH 4, there is no post irradiation increase in iP. But, in 1N NaOH, $G_{(iP)}$ increases to 2.2. Low yields of iP and low yields of labile phosphate esters were obtained when TDP was irradiated in absence of oxygen.

In order to separate the events occurring subsequent to irradiation in oxygen the radiolysis products have been separated by gradient elution from an anion-exchange column. Fractions were monitored for (a) UV absorption, (b) the 2 ^{14}C label of the original TDP and (c) inorganic and labile phosphate. Preliminary results of this investigation indicate that the initial phosphate release occurs from a TDP molecule being damaged in the sugar moiety and releasing both PO_4^- groups. The phosphate ester which breaks down in neutral solution differs from the alkali labile phosphate ester. The former again is a TDP molecule damaged in the sugar group, which, on standing in neutral solution, releases both phosphate groups. The alkali labile phosphate ester is damaged in the base part of the molecule and current experiments are designed to establish the identity of the compound and the mechanism by which it breaks down.

These results lead to some predictions of the mechanisms of strand break production in DNA. 1. The immediate breaks will leave both a 3' and 5' PO_4 end group and eliminate a base moiety. 2. The base moiety eliminated will contain a part of the sugar molecule. 3. The neutral labile phosphate esters lead to similar breaks. 4. Alkali labile breaks are caused by base damage (at least in the case of thymine) - these breaks are apparent only in alkali and are unlikely to be realized *in vivo*.

Radiosensitization by 5-bromouracil: We have previously postulated a mechanism for 5-bromouracil (BU) radiosensitization (Zimbrick et al., Int. J. Rad. Biol. 16, 505, 1969). The hydrated electron, produced by irradiation of water, reacts with BU forming a bromide ion and a uracilyl radical. The latter can react further by abstracting a hydrogen atom from a C—H bond. BU sensitizes because, in contrast to the other DNA bases, it reacts irreversibly with electrons. Two aspects of this mechanism have been further investigated. A. Other radiation induced radicals have been examined for their ability to donate an electron to BU. Reaction with BU was monitored by measuring bromide yields with an ion specific electrode (Orion Model 94-35). The hydrated electron adducts of cytosine and thymine can donate electrons to BU, the reaction is rapid, occurring at a diffusion controlled rate. The radical ions CO_2^- and O_2^- also react with BU. B. The subsequent reactions of uracilyl radicals have been examined: Uracilyl radicals can abstract hydrogen atoms from the deoxyribose moiety of deoxynucleotides. This hydrogen abstraction leads to breakage of the sugar phosphate bond and is therefore equivalent to a DNA strand break. (The uracilyl radical formed by photolysis (257 nm) of BU reacts in a similar way.) These findings suggest that BU is a focus for reducing species damage in DNA and that the damage is in the form of a strand break.

15. EXPECTED RESULTS IN FY 1973

The research discussed in (14) will be continued. Specific areas to be examined are:

1. The alkali labile phosphate esters formed in thymidine diphosphate irradiation.

2. Strand breaks (sb) in irradiated DNA - are the predictions of the model system valid? These areas are of great immediate importance since (a) the existence of base removal as a consequence of strand breakage is not considered in strand break repair mechanisms and (b) most strand break measurements on DNA irradiated in vivo are made under alkaline conditions and many such breaks may not be realized in vivo.

3. Yields of Br^- and sb from DNA which has BU substituted for thymine. The use of various radioactive labels should enable this mechanism to be carefully examined.

Apart from the continuation of this research it is hoped that a new project can be initiated: Currently described enzymatic repair systems recognize gross distortions of the DNA structure. However, damage to some DNA bases could be of a sufficiently minor nature to be undetected by the repair systems - this would constitute a type of damage which could be cumulative. A research project focusing on minor modifications to the adenine moieties of DNA has been designed and it is hoped that this work can commence in 1973.

16. EXPECTED RESULTS IN FY 1974

The extended objective of this program is to develop the work to in vivo systems. All the research discussed will be tested ultimately in vivo. Depending on the outcome of the 1973 program it should be possible to arrive at some conclusions regarding the significance of the various types of damage to DNA.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: PHYSICAL RADIOBIOLOGY

3. AEC Budget Activity No.: 06 01 02

4. Date Prepared: April - 1972

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

6. Working Location: UCLA

7. Person in Charge: E. Hardin Strickland

8. Project Term: From: 1963 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	3	2 1/2	3
(b) Other Tech.	3/4	3/4	3/4
TOTAL:	3 3/4	3 1/4	3 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 44,000	$ 43,000	$ 50,000
(b) Materials and Services	8,500	10,300	11,400
Sub-Total Direct Project Support	$ 52,500	$ 53,300	$ 61,400
(c) Indirect Expenses *	31,600	25,400	26,000
TOTALS:	$ 84,100	$ 78,700	$ 87,400

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

11. PUBLICATIONS DURING FY 1972

Strickland, E. H., M. Wilchek, J. Horwitz, and Carolyn Billups: Effects of Hydrogen Bonding and Temperature Upon the Near Ultraviolet Circular Dichroism and Absorption Spectra of Tyrosine and O-Methyl Tyrosine Derivatives. J. Biol. Chem. 247

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 in England by Aldrich and Cundall and Adams and Wilson. 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. J. F. Ward, and Dr. L. S. Myers, Jr., LNMRB.

14. TECHNICAL PROGRESS IN FY 1972

Progress has been made on 3 different aspects of this project: (A) identifying the effects of hydrogen bonding upon the CD and absorption properties of tyrosyl side chains, (B) theoretical calculations of the tyrosyl CD bands in ribonuclease-S, and (C) the effects of Co^{60}-gamma irradiation upon lysozyme. Each of these areas is described in more detail below.

(A) Effects of Hydrogen Bonding Upon the Near Ultraviolet CD and Absorption Spectra of Tyrosyl Derivatives. To gain information about the properties of tyrosyl residues buried within proteins, the circular dichroism (CD) and absorption spectra of tyrosine derivatives have been investigated in nonpolar

solvents. N-stearyl-L-tyrosine n-hexyl ester dissolved in methylcyclohexane was used to measure the effects of hydrogen bonding agents. Adding low concentrations of dioxane, N,N-dimethylacetamide, n-butanol or methanol causes a 1- to 4-nm red shift in the absorption spectrum and a 10 to 25% increase in the dipole strength. The results with N,N-dimethylacetamide suggest that a hydrogen bond between a tyrosyl hydroxy group and a carbonyl oxygen of the peptide backbone may be one mechanism for producing a large red shift in proteins.

CD spectra were recorded after the hydroxy group of N-stearyl-L-tyrosine n-hexyl ester had been hydrogen bonded. Dioxane and N,N-dimethylacetamide cause the CD spectra to red shift and intensify to the same extent as do the absorption spectra. Evidently hydrogen bonding to these compounds does not alter the conformation of this tyrosine derivative. In contrast, hydrogen bonding of N-stearyl-L-tyrosine n-hexyl ester to butanol or methanol causes a 50% loss of rotatory strength, suggesting an altered conformation. The dependence upon alcohol concentration is the same for both the CD and absorption alterations. Evidence is presented that a polymeric form of the alcohol may simultaneously hydrogen bond to both the hydroxy group and the amide oxygen atom of N-stearyl-L-tyrosine n-hexyl ester.

(B) Interactions Contributing to the Tyrosyl Circular Dichroism Bands of Ribonuclease-S: Within the past several years other investigators have presented theoretical evidence that electric dipole-electric dipole coupling may be a major source of tyrosyl CD bands. I have carried out dipole-dipole coupling calculations (monopole approximation) for the tyrosyl side chains of ribonuclease-S. To obtain the necessary atomic coordinates, the conformation of RNase-S in solution and in glasses at 77°K is assumed to be identical with that reported for the crystalline state by Wyckoff and his coworkers. These calculations reveal several interactions which may give major tyrosyl CD bands in the near ultraviolet region. Approximately half of the experimentally observed CD intensity in RNase-S arises from the interactions between Tyr 73 and Tyr 115. These two phenolic side chains have their hydroxy groups exposed to the solvent. The remaining 4 tyrosyl side chains contribute less than 25% of the CD observed at 275 nm. These results are consistent with our previous suggestion that the disulfide bonds contribute appreciably to the CD of RNase-S between 270 and 310 nm. In RNase-S the side chain of Tyr 25 makes only a small negative CD contribution. Although this phenolic ring is close by the phenyl ring of residue 46, the interactions with the ^1B_b and ^1B_a transitions of the phenyl ring give large tyrosyl CD contributions with opposite signs, which largely cancel each other. A small conformation change, however, would permit this side chain to have an appreciable negative CD band, as is observed experimentally in RNase-A.

(C) Radiation Studies: Previously we observed that the tryptophanyl CD bands of lysozyme (aqueous solution in air) are appreciably diminished by doses as small as 12,000 rads. This irradiation prevents the binding of the competitive inhibitor N-acetyl-D-glucosamine to lysozyme. We are presently attempting to isolate the products of radiation damage so that the individual lesions may be characterized in detail. Thus far 3 fractions have been isolated from irradiated lysozyme. Two of these are minor products at low doses of γ-rays. The two minor components, which lack enzymic activity, have a higher molecular weight than the native lysozyme and a different electrophoretic mobility. The major product has approximately the same molecular weight and electrophoretic mobility as native lysozyme. The enzymatic activity of the major product, however, is only half that of native lysozyme. The degree of heterogeneity of the major product in the irradiated fraction has not yet been determined.

15. EXPECTED RESULTS IN FY 1973

Major attention will be focused upon characterizing the lesions caused by Co^{60} γ-irradiation of lysozyme solutions. The major fraction from irradiated lysozyme (see above) will be examined in more detail. Attempts will be made to fractionate further those species having the same charge density and molecular weight as the native lysozyme. Methods such as affinity chromatography will be used to attempt separations of lysozyme molecules having a reduced ability to bind to substrate analogues and inhibitors. Those fractions which may be isolated will be characterized according to their chemical and spectroscopic properties.

Another approach to identifying the primary site(s) of radiation damage will involve comparative studies with human lysozyme and with chemically modified species of hen egg white lysozyme. The relative radio-sensitivities of native lysozyme and inhibitor complexes will also be examined. Other experiments will be carried out using ion radicals that may react preferentially with tryptophanyl side chains, e.g., Br_2^-. These studies, together with the structure of crystalline lysozyme, may permit identifying the primary sites of radiation damage to lysozyme.

Exploratory studies will be made to examine the conformations of low molecular weight compounds that affect the neural and neuro-muscular systems. For example, CD spectra will be recorded for the prostaglandins available to us ($PGF_{2\alpha}$, PGB_2, PGE_1, PGE_2, $PGF_{2\beta}$).

16. EXPECTED RESULTS IN FY 1974

Investigations will continue generally along the lines proposed for FY 1973. Studies concerning the effects of γ-irradiation will be extended to include other proteins. CD and other spectroscopic techniques will be used to examine the interactions of neural transmitters and related substances with 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.:	4. Date Prepared:
06 02 04	April - 1972

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:
Hideo Nishita	From: 1959 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	3	3	3 1/2
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3 1/4	3 1/4	3 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 46,700	$ 49,300	$ 59,000
(b) Materials and Services	10,500	14,300	17,500
Sub-Total Direct Project Support	$ 57,200	$ 63,600	$ 76,500
(c) Indirect Expenses *	31,600	29,600	30,000
TOTALS:	$ 88,800	$ 93,200	$106,500

* 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 1972

Nishita, H. and Haug, R. M. Some Physical and Chemical Characteristics of Heated Soils. Soil Sci. 113: Accepted. 1972

Nishita, H. and Haug, R. M. Influence of Clinoptilolite on Sr90 and Cs137 Uptakes by Plants. Soil Sci. 114: Accepted. 1972

Nishita, H. and Hamilton, M. Soil Thermoluminescence in Relation to Radiation Exposure under Field Conditions. Soil Sci. 114: Accepted 1972

Nishita, H., Farmer, R., and Peterson, S. An Integrator for Low Absorbance Samples in Atomic Absorption Spectroscopy. Analytica Chemica Acta: Accepted. 1972

Nishita, H. and Hamilton, M. Effects of Some Chemical Treatments of Soils on Their Thermoluminescence. Soil Sci.: Submitted. 1972.

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 products 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. The major emphasis during the FY 1973 will be on the effect of ionizing radiation on soils.

Currently, this Section is involved in the following projects:

(1) Thermoluminescence of soils exposed to ionizing radiation
   (a) Thermoluminescence of soil minerals
   (b) Decay characteristics of soil thermoluminescence
   (c) Methods of using soil as radiation dosimeters
   (d) Influence of chemical treatments of soils on their thermoluminescence.

(2) Availability of essential and nonessential elements to plants grown in soils exposed to heat and ionizing radiation.

(3) Distribution of different forms of nitrogen in desert soils.

(4) Influence of zeolites on Sr90 and Cs137 uptake by plants.

The scope and the objective of these projects are discussed below in the "EXPECT RESULTS" section. Experiments at the laboratory are conducted in the greenhouse and in plant growth chambers. Several soil thermoluminescence experiments will be conducted at the Cs137 radiation field in Rock Valley, Nevada Test Site. Soils will also be exposed to ionizing radiation with Co60 source, a nuclear reactor and the newly acquired cyclotron.

13. RELATIONSHIP TO OTHER PROJECTS

Related research studies within other sections of the Environmental

Radiation Division:

Plant Factors (E. M. Romney, 480311)
Environmental Factors (H. A. Hawthorne, 480320)
Plant Physiological Ecology (A. Wallace, 480344)
Physiology of Mineral Accumulation (O. R. Lunt, 480345)

Related studies at other laboratories:

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

14. TECHNICAL PROGRESS IN FY 1972

The physical and chemical characteristics of five different heated soils were examined. The properties examined include particle size distribution, pH, cation exchange capacity, electrical conductivity, water and ammonium acetate extractable Na, K, Mg, Ca and Sr, and total N, lime and organic C contents. In general, the results of this study showed that soil heating at high temperatures can greatly affect the chemical and physical characteristics of soils. Thus, soil heating could be an important influencing factor on the elemental composition and the growth of plants growing in heated soils.

An experiment was conducted to determine the effect of clinoptilolite on the Sr90 and Cs137 contents of bean and barley plants grown in soils contaminated at the surface (experiment I). An experiment was also conducted to determine the release of Sr90 and Cs137 to clover plants upon continuous and prolonged cropping of a contaminated soil with and without clinoptilolite treatment (experiment II). In experiment I, clinoptilolite was found to decrease the average Sr90 contents of plants. The percentage reduction of Sr90 contents of the different parts of the bean plants ranged from 48 to 70, 54 to 77, and 44 to 77 percent in the leaves, stems and fruits, respectively, depending on the amount of clinoptilolite application and the kind of soil. The percentage reduction of Sr90 in barley plants under various treatments ranged from 57 to 77 and 57 to 79 for the leaves and stems and the heads, respectively. In experiment II, eight harvests of clover were obtained. The application of Ca-treated clinoptilolite was effective in reducing Sr90 contents of the plants grown in the mineral soil used, but it had no effect in the organic soil. Depending on the harvest time, the average Sr90 contents of the plants grown in the treated mineral soil were 59 to 69 percent less than that of the control plants. Clinoptilolite was effective in maintaining the reduced Sr90 contents of the plants throughout the experimental period. The Cs137 contents of the plants were not changed appreciably by the application of clinoptilolite.

Experiments were conducted to determine the relationship of soil thermoluminescence to ionizing radiation exposure under the conditions of natural environment using the Cs137 radiation field at the Nevada Test Site. In experiment I, soil columns were buried in the ground and irradiated at a fixed distance from the Cs137 source. In experiment II, they were exposed on the ground surface at 12 different distances from the source. Soil thermoluminescence was determined in three ways, i.e.,

glow curve area below 200° C, glow curve height at 200° C, and total area. Both experiments showed that soil thermoluminescence is highly correlated with radiation exposure level. Under the experimental conditions that prevailed, the minimum radiation exposure level required to produce measurable soil thermoluminescence appeared to be in the 20-85 R range.

A description of an analog integrator for low absorbance samples in atomic absorption spectroscopy was prepared. This integrator was fabricated in this laboratory. The use of the integrator has improved greatly the instrument sensitivity and the operator readability of the recorder traces of low absorbance samples.

The influence of five chemical treatments of soils on their thermoluminescence was examined. The removal of iron oxides from soils had a markedly great effect in enhancing thermoluminescence. The removal of the carbonates greatly reduced it.

15. EXPECTED RESULTS IN FY 1973

(1) Thermoluminescence of soils exposed to ionizing radiation.
   (a) Thermoluminescence of soil minerals. The objective of this study is to determine the relative thermoluminescent sensitivity of minerals that may be found in significant amounts. Unique thermoluminescent characteristics that may differentiate one mineral type from another will also be sought.
   (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) Methods 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. One method tried already appears to be promising. This method may be used in natural environment exposed to ionizing radiation accidentally.
   (d) Influence of chemical treatments of soils on their thermoluminescence. The objective of this project is to determine whether or not chemical treatments of soils will improve the sensitivity of measuring soil thermoluminescence. Preliminary results have shown that the removal of iron greatly enhances thermoluminescence.

(2) Availability of essential and nonessential elements 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. Two reports on this project have been published (Soil Sci. 110:61-70 (1970); Soil Sci. 113: Accepted (1972)). Presently, work is underway to determine the chemical extractability of Mn, Fe, Cu, Co, Zn and Cr. from heated soils.

(3) Distribution of different forms of nitrogen in desett soils. The objective of this project is to determine the different forms of

nitrogen (NH_4^+, fixed-NH_4, NO_3^-, NO_2^-, and organic-N) in different soils of the Nevada Test Site.

(4) Influence of zeolites on Sr90 and Cs137 uptake by plants. The objective of this project is to determine the relative efficiency of several different zeolites in reducing the Sr90 and Cs137 availability to plants grown in contaminated soils.

16. EXPECTED RESULTS IN FY 1974

Certain aspects of the projects in progress during FY 1973 will be continued. It is anticipated that the soil thermoluminescence studies will be shifted to determine the relative efficiency of different kinds of ionizing radiations (gammas, protons, deuterons, tritons and alpha particles) in inducing soil thermoluminescence. Studies will be made to determine the dependency of soil thermoluminescence on the energy of various ionizing radiation also. This may provide important information in using soil as a radiation dosimeter.

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 - 1972

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 A. Hawthorne

8. Project Term:
From: 1973 To: December 31, 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2 3/4	1 1/2	0
(b) Other Tech.	0	1/4	0
TOTAL:	2 3/4	1 3/4	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 40,400	$ 21,000	$ 0
(b) Materials and Services	9,000	3,600	0
Sub-Total Direct Project Support	$ 49,400	$ 24,600	$ 0
(c) Indirect Expenses *	23,700	12,400	0
TOTALS:	$ 73,100	$ 37,000	$ 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 1972

None

12. SCOPE OF THE PROJECT

The Farm Program arose from a study proposal submitted to the Director's Office in June 1962 and a purchase order, effective July 1, 1962, between UCLA Department of Biophysics and Nuclear Medicine and Mr. Leo Syphus of St. George, Utah. The object of the program was to construct deterministic and stochastic models representing the time course of transport of individual radionuclides from time of injection into the dairy farm through their synthesis into milk. Then relationships affecting differential transfer of the radionuclides were to be elicited by study, integrated with determinations made in the Laboratory, and computer simulations made of system response under perturbation of models. All the data employed in the simulations would come from organisms that were common to the study site, rather than lumping together observations made at different sites, or even data from different countries.

13. RELATIONSHIP TO OTHER PROJECTS

The University of Florida Department of Environmental Engineering has launched a similar program with beef cattle (Dr. J. F. Gamble). The modeling study relates to other projects conducted elsewhere (Ward, Colorado State University; Comar, Cornell University; Pelletier, University of Michigan and others). Collaborative research within the Environmental Radiation Division is with Drs. Beatley and Nishita.

14. TECHNICAL PROGRESS IN FY 1972

All available facilities were utilized in preparation of data tables for the Director's Office. Radionuclide and stable element determinations were combined with measurements collected while feeding trials were in progress and transfer coefficients were computed for the diet-milk transport of eight elements and two radionuclides. Stable strontium and calcium transfer coefficient tables were submitted August 31, 1971, with tables for magnesium, phosphorus, potassium, sodium, rubidium, cesium, and Cs137 submitted at intermediate dates through the year until March 16,1972 when Sr90 tables were completed. Material was prepared for publication from that date until preparation of this Schedule 189. Laboratory work on this program will terminate at the end of FY 1972.

15. EXPECTED RESULTS IN FY 1973

The facilities will be used to prepare manustripts from the data accessible until the termination of the technicians and person in charge during calendar 1972.

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	2 1/2	2	3 1/2
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	2 3/4	2 1/4	3 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 30,600	$ 30,300	$ 42,000
(b) Materials and Services	7,700	13,100	8,400
Sub-Total Direct Project Support	$ 38,300	$ 43,400	$ 50,400
(c) Indirect Expenses *	23,700	17,900	21,800
TOTALS:	$ 62,000	$ 61,300	$ 72,200

* 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 1972

Beatley, Janice C. Vascular plants of Ash Meadows, Nevada. UCLA 12-845. Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles. 59 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 associated project has been the collecting 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 of the world, and radiation effects studies on other AEC-DBM contracts, are more or less pertinent to the total program.

14. TECHNICAL PROGRESS IN FY 1972

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. Soil moisture block and thermocouple installations were removed from the sites in the summer of 1971; useable soil moisture data for the 8 years of measurements await recalibration of the blocks and conversion of the resistance and temperature measurements to soil moisture units (all under the supervision of Dr. Howard A. Hawthorne, with whom the soil moisture project is collaborative). Certain plant data were collected on certain of the sites to meet particular needs for more information, and because of the unusual precipitation regime of the present growing season, it is expected that the winter annual data will be collected again on all of the sites in the spring of 1972.

Vascular Plants of Southern Nye County. An additional 1100 vascular plant collections were accessioned, including around 50 new taxa, bringing the total to over 1100 taxa now known from the region. Around 2100 duplicates were distributed to institutional herbaria and to monographers of the country. Preparation of the final publication will begin in the spring of 1972, with expectations of its completion in the summer; the final publication will include the geographic and ecologic distributions of all of the taxa collected to date, and the citation of all collections upon which the book is based.

15. EXPECTED RESULTS IN FY 1973

Completion of the manuscript dealing with the vascular plants of the region will terminate that phase of the program in progress since 1959.

Preparation of other manuscripts, dealing with the 6-10 years of environmental and biological data for Mojave, Transitional, and Great

Basin ecosystems of the Test Site, continues to be contingent upon the availability of a biostatistician, without which the data analyses cannot proceed.

16. EXPECTED RESULTS IN FY 1974

Data analyses and manuscript preparation, contingent upon a close working relationship with 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: VERTEBRATE RADIOECOLOGY
Formerly Radioecology

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	2 1/2	4 1/4	5 3/4
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	2 3/4	4 1/2	6

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 34,600	$ 63,300	$ 78,000
(b) Materials and Services	8,000	18,800	22,400
Sub-Total Direct Project Support	$ 42,600	$ 82,100	$ 100,400
(c) Indirect Expenses *	23,700	39,600	40,300
TOTALS:	$ 66,300	$ 121,700	$ 140,700

* 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 1972

Continuous irradiated populations. Fundamental Nuclear Energy Research. 1970:: 131-132. (Omitted last year).

Energy flow in southern Nevada populations of Uta stansburiana: a three-year analysis. Abstract in program of annual meeting of Society for the Study of Amphibians and Reptiles, Albuquerque, August 1971. (With P. A. Medica and B. W. Kowalewsky).

Rana pretiosa. Cat. Amer. Amphib. Rept. 119.1-119.4. (With P. C. Dumas).

Estimating lizard home ranges. Herpetol. Rev. 34: 77.

Genetic variation in irradiated and nonirradiated populations of the lizard, Uta stansburiana. Radiat. Res. 47: 530-536. (With C. O. McKinney).

Lizard sampling techniques. Rock Valley Misc. Publs. No. 1 (P. A. Medica, G. A. Hoddenbach, and J. R. Lannom, Jr.).

Radiation-induced sterility in natural populations of lizards (Crotaphytus wislizenii and Cnemidophorus tigris). Proc. Third Natl. Symposium on Radioecology. (With P. Licht, J. D. Thrasher, P. A. Medica, and J. R. Lannom, Jr.).

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 sub-lethal 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 population 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 date 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 1972

1. The development of sterility among irradiated female leopard lizards (Crotaphytus wislizenii) and whiptail lizards (Cnemidophorus tigris) has been discussed previously. This condition has also apparently developed among female horned lizards (Phrynosoma platyrhinos). The numbers of horned lizards observed in the three fenced areas in Rock Valley clearly indicate a difference between the irradiated plot (2) and the other two control areas. In the following tabulation the three columns for each area represent, respectively, numbers of adults (≥ 20 months), yearlings (8 months), and summer hatchlings. (Spring populations are, then, the totals of the first two columns.)

	Area								
Year	1			2			3		
1964	12	2	0	7	5	4	10	10	5
1965	8	0	6	8	3	0	13	4	12
1966	4	5	5	8	0	1	6	6	24
1967	7	5	11	5	1	0	10	18	5
1968	10	11	18	6	0	0	19	4	14
1969	13	16	46	2	0	2*	15	8	31
1970	21	32	0	1	2*	0	12	20	0

*Believed to be immigrants.

Our conclusion from the data summarized above is that continued gamma irradiation has essentially blocked reproduction by Phrynosoma platyrhinos in the irradiated plot since about 1966 or 1967. Between the inception of the experiment in 1964 and 1966 the demographic evidence is somewhat equivocal, but from the summer of 1967 to 1970 (and particularly in 1968 and 1969) the contrast between the two control plots and the irradiated plot is striking. Reproductive failure in the irradiated area is clearly manifested by the virtual absence of summer hatchlings and spring yearlings, and by the decline in older individuals during a time when nonirradiated populations were increasing. Furthermore, one of the resident female Phrynosoma in the irradiated plot was collected during the spring of 1971 and a laparotomy performed. This individual showed the same absence of ovarian tissue and hypertrophy of fat bodies already described among whiptail and leopard lizards. Although the first appearance of sterility among females of the horned lizard population cannot be firmly established, the sampling data suggest 1966-67. This is about the time that sterility was first manifested in the irradiated leopard lizard population. Previously we have emphasized the apparent lack of radiation effects on Uta stansburiana, and explained this in terms of the early maturation and short life-span of this species. Because in the longer-lived lizards about three years of radiation exposure resulted in regression of ovaries, it seemed possible that Uta females might be spared this effect simply because of their shorter life-span. However, in 1971 three female Uta 22-33 months of age were taken from the irradiated plot and autopsies showed the same ovarian regression and fat body hypertrophy previously observed in the other species. Later in the spring 110 female Uta (of unknown age) in the irradiated area were examined by palpation. It was estimated that 30% of these females were sterile. This proportion was about the same as the proportion of females 20 months and older (25%) among Uta of known age. There is, then, both direct and indirect evidence to indicate that radiation-induced sterility may develop in Uta within 20 months. It also seems likely that this condition has existed from some years but was simply not detected. In a three-year analysis of irradiated and nonirradiated Uta populations (1966-1968) we examined spring age-distributions and sex ratios, as well as annual density changes. We observed no difference between the population in the irradiated area and the three control areas. Yet present evidence indicates that egg production by irradiated older females was very likely reduced during these years.

These facts could be reconciled if there were differences in juvenile survivorship between the time of hatching in the summer and the following spring. That is, reduced egg production by the irradiated population might be compensated by better relative survival among those young produced. If this occurred, the composition of spring populations (as examined in the 3-year study) would reveal no apparent differences between areas.

Minimal survival of juvenile Uta in the four study areas for the five years 1966-1970 was analyzed. Initial rosters of young Uta (N) were made up of animals originally registered between July 1 and August 31 and $\leq$ 28 mm in snout-vent length at time of marking. Survivorship was estimated from the number of individuals (S) of these initial cohorts recaptured at any time the ensuing spring (i.e., after March 1).

Whereas this measure of minimal survival underestimates true survival to whatever degree emigration has occurred, the sampling procedures were similar in all four areas. The results of this analysis show a startling difference between minimal survival of female hatchlings in the irradiated area and in the other three plots:

	Areas 1, 3 and 4			Area 2		
Year	N	S	8-month survival	N	S	8-month survival
1966	128	20	0.16	36	9	0.25
1967	126	24	0.19	50	16	0.32
1968	130	26	0.20	34	8	0.24
1969	125	24	0.19	43	21	0.49
1970	69	17	0.25	31	14	0.24
5 years	578	111	0.19	194	68	0.35

Five-year mean survival in area 1 was 0.19, in area 3:0.18, and in area 4:0.23. A comparable analysis of male hatchling minimal survival yielded 5-year means of 0.22 in the irradiated area and 0.12 in the other three areas. The apparent difference between sexes is probably due to greater mobility among young males.

If the improved hatchling survivorship in the irradiated area is real it acts to compensate for the reduced egg production owing to sterility among older females. On average, yearling females produce about 70% of the eggs laid each spring, so a population in which older females were sterile would produce correspondingly fewer young. However, an improvement of hatchling survival on the order of 40% would compensate for the impaired fertility of the older females. The observed difference in hatchling survival is considerably greater than this, owing mainly to the very high survivorship observed in 1969-70 and 1970-71. The main point to be made at this time is that the demographic parameters of natural populations may be flexible, and stresses below some critical threshold can be met by the development of new equilibrium states.

2. Rodents were trapped in the three fenced enclosures in Rock Valley during the spring (April-May) and again during the summer of 1971. Early in the Rock Valley experiment (1965) both species of kangaroo rats (Dipodomys merriami and D. microps) disappeared from the irradiated plot. This was not attributed to radiation, but rather to the initial low densities coupled with extremely unfavorable conditions during the 1964 breeding season. Trapping in 1971 revealed that both of these species were again present in the irradiated area. Their occurrence there was apparently due to immigration when the fence was not rigorously maintained during 1969 and early 1970. In the spring 13 Dipodomys microps were trapped in the irradiated area, while 4 and 2 individuals were taken in the other two fenced plots. At the same time 8 D. merriami were taken in the experimental area, and four were captured in one control area and none in the other. The abundance of Perognathus formosus in the irradiated plot is still apparently normal. In the spring of 1971, 51 individuals were trapped, while in the two control plots 42 and 81 were taken. Densities of heteromyids in all plots were generally

less than those observed in 1970. Reproduction in 1971 was not good. No juvenile P. formosus were captured in the irradiated plot and in one control area; only two were taken in the other control plot. Among heteromyids there were no manifestations of radiation effects comparable to those reported for lizards.

3. Observations of birds in Rock Valley began in two fenced 8-ha plots in March 1971, and was continued into June. Observations were made again during November and December. One of the plots (2) was irradiated and the other was an adjoining control area.

Spring breeding bird densities were estimated by the Williams spot map census technique. Observations were made along parallel lines 50 m apart. Fifteen to 19 censuses were taken over a four-month period during the spring. Non-breeding species (transients, winter visitants), whether singing or not, were also tallied in the course of each census. Nest searching augmented regular census techniques for species lacking strong territorial behavior or distinctive song.

Estimated densities of the two breeding species are given below:

Species	Plot	Pairs	Pairs/ha	g/ha
Toxostoma lecontei	1	0.25	0.03	3.76[1]
	2	0.25	0.03	3.76
Amphispiza bilineata	1	4.00	0.50	13.40[2]
	2	4.25	0.53	14.31

[1] assuming a mean body weight of 62.7 g

[2] assuming a mean body weight of 13.4 g

Counts of non-breeding birds are given below:

Species	Minimum visiting period (weeks)	Plot 1 March	Plot 1 April	Plot 1 May	Plot 1 June	Plot 2 March	Plot 2 April	Plot 2 May	Plot 2 June
Zenaidura macroura	1			3(5)				6(5)	
Phalaenoptilus nuttallii	1		0(5)				1(5)		
Tyrannus verticalis	1			1(5)				1(5)	
Sayornis saya	1	0(3)				1(3)			
Tachycineta thalassina	4	0(2)	0(2)			1(2)			
Mimus polyglottos	5		1(7)	0(3)			0(5)	2(3)	
Phainopepla nitens	1			1(4)				0(4)	
Dendroica petechia	1			2(3)				2(3)	(contd.)

Counts of non-breeding birds (contd.)

Species	Minimum visiting period (weeks)	Plot 1 March	Plot 1 April	Plot 1 May	Plot 1 June	Plot 2 March	Plot 2 April	Plot 2 May	Plot 2 June
D. auduboni	5		3(7)	1(2)			7(5)	0(2)	
Wilsonia pusilla	1			0(5)				1(5)	
Icterus parisorum	4		0(2)	0(3)			1(2)	0(3)	
Euphagus cyanocephalus	1		1(7)				1(4)		
Carpodacus mexicanus	9		3(8)	2(5)	0(2)		3(5)	2(5)	1(5)
Amphispiza belli	1	5(3)				6(3)			
Spizella breweri	2		11(9)				6(5)		
Zonotrichia leucophrys	6	1(3)	7(9)			9(3)	9(5)		

For the transient species the total numbers of individuals counted during all of the censuses are given. Fringillids dominated the fauna during March and April, but declined in relative importance during May. Other species of birds observed in the plots during March and April were: Cathartes aura, Falco sparverius, F. mexicanus, Corvus corax, Circus cyaneus, and Speotyto cunicularia. Of these the raven was the most commonly observed.

4. Energy flow of Uta stansburiana occupying an 8-ha area in southern Nevada was estimated over a three-year period between March 1, 1965, and February 29, 1968. Analysis was restricted to assimilated energy and did not include energy ingested but not utilized. Energy of respiration (R) and elimination (E), as well as changes in standing stock (ΔB) were estimated for monthly intervals over the three-year span. Production (P) was considered to be $E + \Delta B$. The basic computer simulation of energy utilization took into account daily changes in numbers and body weights of lizards (on an age- and sex-specific basis), as well as maximum attainable body temperatures (T_b). For each day, oxygen consumption was estimated hourly for each sex and age group by equations of the form: $cc\ O_2 = \frac{XW^Y Z^T}{}$, with W = body weight and $T = T_b$. Allowances for metabolic scope were built into the simulation. During any time period, E was equal to the caloric equivalent (1.567 Kcal/g) of all Uta dying. Estimated annual energy flow ranged from a low of 1792 Kcal/ha in 1965-6 to a high of 2719 Kcal/ha in 1966-7. Energy of respiration ranged from 1458 to 2184 Kcal/ha and production from 336 to 536 Kcal/ha. In terms of the ratio of production to respiration (P/R) Uta was functionally analogous to other ectothermic animals (invertebrates, fish). Annual variations in P/R ranged from 23 to 35% and seasonal fluctuations of from 8 to 57% were observed. Annual fluctuations in energy parameters were closely tied to changes in population composition and density, as well as to differences in growth rates of juveniles. The basic simulation was modified to allow for different assumptions as to rates of death, growth and metabolism as well as different patterns of metabolic scope. Knowledge of changes in density owing to mortality and reproduction were

indispensable to any type of analysis, but in this particular study the factor of greatest importance was the choice of physiological submodel. Using alternative measurements of minimum metabolic rates resulted in estimates of annual metabolism 3-4 times higher and of annual energy flow about 3 times higher than those obtained with the basic simulation. No obvious rationale is available for selecting between these alternatives. Another objective of this research was to test the utility and reliability of simplified methods of estimating energy utilization parameters. Values of respiration, elimination, production and energy flow were regressed on various population state variables. Production and elimination were most significantly correlated with biomass values. Energy of respiration was highly correlated with densities. For the three years in question annual energy flow (Kcal/ha) could be expressed as 2.2 (April + November biomass in g/ha) + 16$\bar{N}$ + 102.3, with $\bar{N}$ the average daily density/ha for each year. Three models for estimating $\bar{R}$ were tested and one of these, based on mean monthly densities, yielded estimates of annual adult respiration within 5% of those obtained with the basic simulation. This model failed, however, to distribute the annual energy of metabolism accurately within months. Our findings indicated that Uta stansburiana, and possibly other numerous and short-lived lizards, are of greater significance in the energy dynamics of natural communities than has heretofore been appreciated. It is also possible to develop relatively simple models of Uta energy flow (including $\bar{R}$, $\bar{E}$, and $\Delta\bar{B}$) based on monthly changes in population state.

5. During the spring of 1972 a study of the effects of several experimental manipulations on reproduction and survival of Uta stansburiana was begun. This study is designed to be conducted over a period of two years and involves the use of six one-acre enclosures. Treatments will involve artificial irrigation (by sprinkling) during the fall, density modifications, and predator removal. During the fall of 1971 the equivalent of 2 inches of rain were added to two of the six one-acre enclosures. In the spring of 1972 densities of 0, 1 and 2 leopard lizards were established in each of two plots. At the same time densities of Uta were artificially reduced in half the plots and increased in the other half. Over the course of a two-year period we will examine 12 combinations of rainfall, predation and density. For each situation we will measure i) annual survival rate of 8-month-old females, ii) annual survival rate of older females, iii) survival of young (born during the summer) to the ensuing spring, and iv) average egg production per female alive March 1.

15. EXPECTED RESULTS IN FY 1973

1. An important aspect of continuing research in Rock Valley will have to do with changes in the irradiated community as this system moves towards a new equilibrium arising from the selective extinctions of more sensitive species. We have already identified two species of lizards which are disappearing from the irradiated plot, and two other species in which reproduction has been impaired. One sterile female of a fifth species (Coleonyx variegatus) has been collected from the experimental plot. What indirect effects may arise as a result of these effects? To what degree can compensatory changes in survival

resist female sterility? We have commented on this situation in Uta and given specific data relating to apparent compensation of this nature. To what extent are such modifications occurring in other species? The situation in rodents needs renewed attention, particularly in view of the fact that the oldest females in some of the populations have received radiation doses as large as those known to be sterilizing in lizards.

2. State measurements of vertebrate populations on the validation site will be continued, together with estimates of standing stocks and conventional measures of species diversity.

3. The experimental manipulation studies involving Uta stansburiana and Perognathus formosus will be continued.

The observations on pocket mice should be relevant to the interactions of rodent and plant populations on each other's processes: e.g., how shrub density affects rodent population dynamics and diversity; how a high density has a feedback effect on a population; the effect of annual production on rodent reproduction; the effect of rodent species diversity on rodent processes. All of these are important to a predictive model of a desert ecosystem. The main value of an enclosure is that natality and mortality can be differentiated from immigration and emigration. The artificial barring of emigration may explain the higher than natural densities observed in 1- and 2-acre enclosures by Pomeroy and Gentry in Georgia. The few studies that have been done to date on mammals within enclosures are encouraging. Barrett found that the responses of rodents to a change in the system can be detected in 1-acre plots; he observed a delayed reproduction by Sigmodon hispidus after insecticide spraying of an old-field enclosure, but not in an immediately adjacent control enclosure. Caldwell and Gentry's observations on the interactions of Mus and Peromyscus in 1-acre enclosures corroborated their observations and conclusions with the same species in open-field studies. Mus cannot survive with Peromyscus when it is unable to shift its home range with changes in food conditions. When there is a non-fatal negative response to a manipulation, such as, possibly, a dispersal from a shrubless area, this could not be expressed in an enclosure.

4. An important expansion of the program is foreseen in FY 1973, involving the addition of a new staff member with interests in the ecology of rodent populations and the physiological adaptations of individual animals to desert environments. Dr. Kenneth Nagy, presently of the Zoology Department at the University of California, Los Angeles, is visualized in this position. In addition to Dr. Nagy, appropriate technical support will be required. These expansions account for most of the increase in budgetary support between FY 1972 and FY 1973.

16. EXPECTED RESULTS IN FY 1974

The program in FY 1974 will be largely focused in Rock Valley, with the major problems being:

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

b. maintenance of a system for monitoring meteorological variables, together with procedures for retrieval and reduction of data
c. maintenance of retrievable data sets, and the periodic transmission of information to the I.B.P. central office management at Utah State University
d. active participation in the development of process and systems models by means of collaboration with the modeling group at Utah State University.

In FY 1974 it is expected to make a new appointment in this group, possibly a person with experience in the dynamics of insect populations and with a capability in modeling ecological processes. This addition results in an increase in requested budget support between FY 1973 and FY 1974.

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: ANALYSIS OF ECOSYSTEMS
Formerly Radiation Ecology

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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: 1959 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	1 ½	2 ½	3
(b) Other Tech.	¼	¼	¼
TOTAL:	1 ¾	2 ¾	3 ¼

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 20,200	$ 37,300	$ 45,000
(b) Materials and Services	6,100	14,200	17,100
Sub-Total Direct Project Support	$ 26,300	$ 51,500	$ 62,100
(c) Indirect Expenses *	15,800	21,800	23,500
TOTALS:	$ 42,100	$ 73,300	$ 85,600

* 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 1972

Publications related to I.B.P. endeavors are listed under the various sections in the Division.

12. SCOPE OF THE PROJECT

A major goal of the International Biological Program is to develop understanding of the biological basis of productivity in nature. This implies a comprehension of the dynamics and functioning of the ecosystems into which natural processes are integrated. If this understanding is sufficiently profound, it can serve as an effective guide to prediction of the changes that are likely to come about when ecosystems are subjected to stresses and/or manipulations, and hence to direct the wise use and management of natural resources. Applying this general objective to Rock Valley and the Nevada Test Site, we may express our immediate objectives as follows:

1) to elucidate productivity, nutrient cycling and energy flow in a desert ecosystem,
2) to determine the processes involved in the transfer of matter and energy among the components of this system, and
3) to synthesize the results of these studies into predictive models to serve as guides in the management of deserts in general and in the understanding of the impact of radioactivity on arid environments.

13. RELATIONSHIP TO OTHER PROJECTS

The validation site in Rock Valley, adjoining the 20-acre fenced areas, is a part of the Analysis of Ecosystems Program of the U.S. International Biological Program. In a broad sense, then, our research is related to comparable endeavors in other biomes: grasslands (Colorado), deciduous forests (Tennessee, Wisconsin, New York), tundra (Alaska), and coniferous forests (Washington). Specifically, our site is a part of the desert biome program. Our contribution to this work involves periodic measurements of the state of various plant and animal populations occupying a 1/2-km^2, together with regular monitoring of meteorological and soil state variables. These measurements will ultimately be used in the testing and improvement of models of system function being devised at Utah State University. Involvement in desert biome research has fostered a close relationship between the biologists in our Division and the modeling team (mathematicians, programmers) at Utah State. Data acquired in Rock Valley are being made available to the biome central management at Utah State. Our work in Rock Valley is also related to that being conducted at three other validation sites: Curlew Valley in northern Utah and southern Idaho, Avra Valley near Tucson, and the Jornada Experimental Range near Las Cruces, New Mexico. The validation site program in Rock Valley has also led to collaborative programs with biologists at the University of Southern California, Long Beach State University, and the University of California at Riverside.

14. TECHNICAL PROGRESS IN FY 1972

Progress during FY 1972 is discussed under the accounts of other sections in the division. The vegetation of the IBP validation site was analyzed in considerable detail during the spring and summer of 1971 (Quantitative

Plant Ecology: Wallace-Bamberg), both with respect to the state of the perennial shrubs and in terms of production of winter annuals during the spring. This work was supported by measurements of abiotic influences and phenological observations, as well as the development of regressions relating shrub biomass to linear dimensions (Plant Factors: Romney). At the same time chemical and physical analyses of the soils of the validation site were carried out (Soil Survey and Characterization:Hale). Analyses of elemental composition of plant and animal samples by emission spectroscopy continued (Distribution and Interrelationship of Elements in Biological Systems: Alexander). In addition to the validation measurements relating to plant populations, two studies of important ecological processes were begun: measurements of photosynthetic and respiratory rates of various shrubs (Quantitative Plant Ecology: Wallace-Bamberg) and analysis of the growth and distribution of roots of desert shrubs (Plant Physiology and Ecology: Wallace). Work on animals involved pitfall and vacuum sampling of arthropods (Desert Arthropods: Edney), censuses and counts of breeding and non-breeding birds, and continuing assessments of populations of reptiles and mammals (Radioecology: Turner). Additionally, two studies of vertebrate demographic processes were begun: one on Uta stansburiana and one on Perognathus formosus (Radioecology: Turner).

15. EXPECTED RESULTS IN FY 1973

Validation site measurements of plant and animal populations will continue (Nutrient and Radionuclide Cycling: Romney, Desert Arthropods: Edney, Radioecology: Turner). By arrangement with Utah State University, spectroscopic analyses of biological samples will be expanded to include material from other areas throughout the biome (Distribution and Interrelationship of Elements in Biological Systems: Alexander). Quantitative analyses of selected ecological processes will continue (Quantitative Plant Ecology: Wallace-Bamberg, Radioecology, Turner).

16. EXPECTED RESULTS IN FY 1974

Validation site measurements will be phased out but work on ecosystem analysis may continue (with extramural support) with emphasis on the modeling of particular ecological processes.

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	5	5	5 1/2
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	5 1/4	5 1/4	5 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 59,000	$ 60,300	$ 67,000
(b) Materials and Services	14,500	22,400	25,200
Sub-Total Direct Project Support	$ 73,500	$ 82,700	$ 92,200
(c) Indirect Expenses *	48,900	36,900	34,400
TOTALS:	$ 122,400	$ 119,600	$ 126,600

* 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 1972

The Carrier-Free Isolation of Indium from Silver and Cadmium by Liquid-Liquid Extraction. J. Inorg. Nucl. Chem. (In press)

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 in this division and other divisions. These programs have involved the preparation and analysis of samples for specific radionuclide content as well as the determination of trace elements using activation analysis and other instrumental techniques.

The primary effort during the past 8 months has been in the area of stable element analysis using neutron activation analysis techniques extensively. In addition, this section has been involved in the production, isolation and purification of short-lived isotopes associated with the recently installed medical cyclotron.

The analysis of fission products by wet radiochemical techniques is limited entirely to Sr^{90}, all other nuclides are being determined by gamma pulse height analysis.

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 1972

During the first 8 months of FY 1971 this section completed the analysis of several hundred plant samples for stable Cl and S. In addition, an equal number have been analyzed for stable Cs in support of division research programs.

A number of rapid carrier-free liquid extraction methods were developed for the isolation and determination of various fission products as well as short-lived radionuclides produced by charged particle activation. A number of new chelate complex of HDEHP have been isolated and are presently under investigation.

15. EXPECTED RESULTS IN FY 1973

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 considerable effort will be made to develop the capability of analyzing plant and animal tissue for N^{15} and O^{18} employing an activation method using cyclotron-produced charged particles.

16. EXPECTED RESULTS IN FY 1974

It is anticipated that during the FY 1974 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 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: DISTRIBUTION AND INTERRELATIONSHIP OF ELEMENTS IN BIOLOGICAL SYSTEMS

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	1	1	1
(b) Other Tech.	0	0	0
TOTAL:	1	1	1

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 14,000	$ 14,000	$ 14,000
(b) Materials and Services	2,900	3,900	3,900
Sub-Total Direct Project Support	$ 16,900	$ 17,900	$ 17,900
(c) Indirect Expenses *	6,000	6,100	6,700
TOTALS:	$ 22,900	$ 24,000	$ 24,600

* 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

Wallace, A. and Collaborators: "Regulation of the Micronutrient Status of Plants by Chelating Agents and Other Factors." A. Wallace, editor. 309 pp. (1971).

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 an emission spectrometric analytical system has been developed which requires little sample preparation, is simple to operate and is capable of determining some 25 elements commonly observed in plant and animal tissues. This system is being used for routine analysis at present but, with the addition of several refinements, will remain in a developmental state for several years to come.

13. RELATIONSHIP TO OTHER PROJECTS

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

14. TECHNICAL PROGRESS IN FY 1972

The optical emission system was completely overhauled during the first six months of this year with the addition of fifteen data channels and a new solid state controller. Analytical channels were added for the elements beryllium, mercury, arsenic and antimony. This addition necessitated a complete revision of the slit/phototure array. Detectors for several molecular species useful in estimating background signals were added to the system. Preliminary results indicate that the overall performance of the spectrometer system has been improved.

15. TECHNICAL PROGRESS IN FY 1973

In addition to the continuing analysis of plant and animal tissues at the rate of approximately 10,000 per year much time and effort during FY 1973 will be devoted toward the improvement of the calibration procedures.

16. EXPECTED RESULTS IN FY 1974

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: ECOLOGY OF DESERT ARTHROPODS

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
Eric B. Edney

8. Project Term:
From: 1973 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	0	2	2 1/2
(b) Other Tech.	0	1/4	1/4
TOTAL:	0	2 1/4	2 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 0	$ 43,200	$ 46,000
(b) Materials and Services	0	13,100	13,900
Sub-Total Direct Project Support	$ 0	$ 56,300	$ 59,900
(c) Indirect Expenses *	0	25,400	23,500
TOTALS:	$ 0	$ 81,700	$ 83,400

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

11. PUBLICATIONS DURING FY 1972

Edney, E. B. The body temperature of tenebrionid beetles in the Namib Desert of southern Africa. J. Exper. Biol. 55: 253-272.

12. SCOPE OF THE PROJECT

The ecological importance of arthropods in desert systems is only just beginning to be studied, and it is the overall aim of the present project to contribute to this field of investigation, with particular regard to the situation at the Nevada Test Site.

First, it will be necessary to identify certain species as being of particular significance in terms of their contribution to the maintenance of the ecological system. (Grasshoppers, leafhoppers, mirids, termites, and scorpions are obvious candidates.) One or more selected species will then be studied intensively to determine the effect of abiotic factors, particularly water and temperature, upon their distribution and abundance. Finally the effects, if any, of ionizing radiation on the physiological well-being, and hence on the ecological competence, of the species concerned will be studied. The above program is, of course, a long-term one, and will have to be broken down into several more identifiable, specific problem areas, and this has been done in section 15 below.

13. RELATIONSHIP TO OTHER PROJECTS

The survey of arthropods associated with shrubs (see section 14 below) is related to other surveys of plants, reptiles and mammals that have been, or are being, made at the Nevada Test Site. A search for effects stemming from long continued exposure to gamma radiation is similarly related to studies by Drs. Turner and French of this laboratory on lizards and rodents respectively.

Work on the water and energy budgets, including food consumption, of arthropods is a necessary component part of the overall approach to desert ecology being undertaken by this Laboratory. It also pertains to the invertebrate studies being undertaken by the I.B.P. desert biome program in the Chihuahuan, Sonoran and Great Basin deserts. Such work also relates to basic studies of desert arthropod physiology being done at the Universities of Arizona and New Mexico, and at other institutions. Information about consumption rates and energy flow will also be relevant to the work on radioecology of arthropods being conducted at Oak Ridge.

14. TECHNICAL PROGRESS IN FY 1972

Since the program is largely new, the only progress to be reported for the current year is that achieved by Dr. Elbert Sleeper of Long Beach State University in his I.B.P.-supported investigation of terrestrial and shrub-dwelling arthropods, and a brief report of his work follows.

As a part of the overall arthropod investigations at Rock Valley, Nevada Test Site, a study of the fenced plots 1 (nonirradiated) and 2 (irradiated) was undertaken. The object of the study was to determine whether there were any significant differences between the arthropod faunas of the two

plots after several years of radiation.

Sampling was done by the standard vacuuming methods suggested by Bender and MacMahon (1971) for desert shrubs. For terrestrial or "walking" forms, we used the previously placed pitfall traps originally designed for reptilian studies.

Vacuum samples were taken in plots 1 and 2 on alternate weeks over a period of 22 weeks. This schedule was dictated by the necessity to take samples in plot 2 when the source of the radiation was down. A total of 108 samples (ca. 10 in each 2 weeks) were taken at predetermined intervals in each plot. The shrubs were sampled in accordance with their relative abundance. For example, in plot 1 sampling effort among the five most important shrubs was distributed approximately as follows: Larrea divaricata (27 percent), Franseria dumosa (19 percent), Lycium andersoni (20 percent), Ephedra nevadensis (10 percent), and Krameria parvifolia (17 percent). Further sampling was distributed among shrubs of lesser importance. In plot 2 the effort was nearly identical (± 1 percent).

Within a day or two of taking the vacuum sample, 100 pitfall traps were inspected; arthropods were collected, recorded, selected species marked, and released. In plots 1 and 2, 2000 and 2100 trapnights respectively were accumulated (ca. 200 trapnights/week/plot). The marked specimens will be recaptured and the accumulated data used to estimate populations.

With only one year of sampling completed and the data only partially analyzed, it is somewhat premature to draw many conclusions. However, some trends seem evident. The species found in the pitfall traps do not seem to be affected by radiation (if this is really the only major difference between the two plots). This seems to apply equally to the predators and phytophagous species. For instance, our most abundant spider, Psilochorus utahensis (Pholcidae), varies insignificantly. In May the difference per hundred traps was less than 2/100 trapnights (50 for plot 1; 48 for plot 2); in early July the difference was 4/100 trapnights (62 in plot 1; 58 in plot 2). One month later in early August the trend was reversed: 6/100 trapnights (70 in plot 2; 64 in plot 1). Comparable figures were found for scorpions and the scavenging Tenebrionidae. Rain at an opportune time seemed more important, as numbers increased dramatically in both plots afterwards. On the other hand, significant differences were found in the shrub-dwelling forms. So far, analysis of the phytophagous species shows that those that are soft-bodied are significantly different between the two plots. For instance, the numbers of the grasshopper Bootetix punctata are uniformly three to five times larger in plot 1 than in plot 2. The same trend was found in the scale insects (Pseudococcidae) and the thrips. However, the trend did not apply to the Membracidae on Larrea. Here there appears to be no significant difference between plots. The harder bodied forms (Coleoptera), as intimated above, show very little, if any, difference (Chrysomelidae on Larrea and Franseria), as numbers varied by less than 1% for weekly population levels. This seems to apply also to the phytophagous Tenebrionidae and Curculionidae.

15. EXPECTED RESULTS IN FY 1973

a) Information in a somewhat undigested form, derived from can-trapping studies some years ago, is available, and this will be organised and used to help in identifying important species. Further information will be obtained from the continuing I.B.P.-supported shrub arthropod program, and in addition automatically timed traps will be used to provide more precise information about the distribution of activity of various ground species throughout daily and annual cycles.

b) Investigations on the effect of water and temperature on the distribution and abundance of ecologically interesting species will form an important part of the program. Relationships will be sought between water and heat on the one hand and reproduction, development and mortality on the other. Field work will include measurements of microclimates and contemporaneous recording of behavior of the animals in their habitats. Laboratory studies will attempt to analyse behavioral responses, particularly insofar as these affect the location and movement of the animals within their habitat and thus the microclimates to which they are exposed.

The effect of water and heat on reproduction, development and mortality will be studied in the laboratory. However, an important part of the work will be an attempt to obtain information about water and energy budgets of animals in field conditions. Techniques for doing this with vertebrates (using tritium and ^{18}O) have been developed, and their extension to arthropods should be productive. By loading individuals with a measured tritium concentration and following the changes in this with time, it should be possible to measure the extent of each significant source of water gain and loss, including the amount of pre-formed water taken in with food. If the water content of the food is also known, the amount of food taken can be calculated. This, combined with knowledge of the energy content of the food and of the excreta, yields information about the energy budget of the species. Laboratory studies to develop the necessary techniques will be made, using desert arthropods, and the results will be applied in the field.

c) A third part of the program concerns the effects of ionizing radiation on arthropods. There is little information about beta radiation effects, and since arthropods are small, and a large part of 'fallout' radiation energy is in this form, measurement of possible effects of beta radiation on desert species is indicated. The present program will be concerned in the first place with the effects of radiation on water balance mechanisms, because hard beta radiation is known to affect water balance profoundly in domestic cockroaches. Beta dosages will be administered by feeding insects on contaminated food and by surface application.

Possible effects of long continued exposure to relatively low levels of gamma radiation will be looked for in insects in Rock Valley by examining the reproductive organs and performance of long-lived insects (such as tenebrionid beetles) and of grasshoppers which have been subjected to such treatment in the course of the irradiation experiment.

16. EXPECTED RESULTS IN FY 1974

Studies on the significance of water and heat on the distribution and abundance of selected species will be expanded in field and laboratory conditions. Studies on the effects of radiation will begin.

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: NUTRIENT AND RADIONUCLIDE CYCLING

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
Evan M. Romney

8. Project Term:
From: 1973 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	0	8 1/4	11 1/4
(b) Other Tech.	0	1/4	1/2
TOTAL:	0	8 1/2	11 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 0	$ 118,300	$ 147,000
(b) Materials and Services	0	31,000	37,800
Sub-Total Direct Project Support	$ 0	$ 149,300	$ 184,800
(c) Indirect Expenses *	0	68,900	75,600
TOTALS:	$ 0	$ 218,200	$ 260,400

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

11. PUBLICATIONS DURING FY 1972

Listed in other sections.

12. SCOPE OF THE PROJECT

The general objectives of the Nutrient and Radionuclide Cycling project encompass research activities in the following areas of investigation:

1. Ecosystem radionuclide cycling, including radiation effects on plants.

2. Mineral nutrient cycling, including nitrogen and non-essential heavy metals.

3. Carbon cycling with emphasis on CO_2 exchange, decomposition, and seasonal retranslocation.

4. Water cycling with special interest in desert conditions.

Within these general areas of investigation are several interacting studies of mutual need which form the technical basis of this research program. Included are the following:

a. Influence of abiotic factors, including the monitoring of environmental conditions in field study plots at the Nevada Test Site.

b. Plant characterization in the desert ecosystem, including patterning, association and distribution, as influenced by nutrient gradients and other edaphic factors.

c. Root growth and distribution with special emphasis on ion uptake mechanisms and water utilization.

d. Recovery of disturbed environments (fallout, natural, technological), including the artificial revegetation of areas having slow natural recovery.

e. Effects of environmental manipulations in the desert ecosystem, including the impact of radiation, heavy metal toxicity, fertilization and irrigation.

f. Soils characterization.

These research activities are part of a team program involving a systems analysis approach to an understanding of the ecological attributes and functions of plants and soils 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 the dissemination of radioactive contamination into the environment and to help increase understanding of the functional and homeostatic factors involved in maintaining the integrity of desert vegetation. These studies should help establish parameters for assessing the ecological consequences resulting from technological disturbance and radionuclide contamination of the desert environment. 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 US/IBP Desert Biome Program.

Some unique analytical systems and support facilities are utilized by this team research project. These include an automated emission spectrometer for mineral element analysis of plant and animal tissues; the UCLA Engineering Reactor and Medical Applications Cyclotron for activation analysis work; an automated Siemens null-point compensation system for in-field gas exchange studies; access to fallout contaminated areas for ecosystem radionuclide cycling studies, including plutonium problems at the Nevada Test Site; and the tower-mounted Cs137 radiation source in Rock Valley for studies of long-term gamma irradiation effects.

13. RELATIONSHIP TO OTHER PROJECTS

Research activities are correlated with the work of other sections in the Environmental Radiation Division. Related studies are conducted at the Biology Department, Battelle-Northwest Laboratories; Radiation Ecology Section, ORNL; Biology Department, BNL; Biology Division, LLL; Sections H4 and H7, LASL; E.G. & G., Santa Barbara; Western Radiological Health Laboratories, Las Vegas; Biology Department, Emory University; and US/IBP Desert Biome Program, Logan, Utah. Several off-site contracts to various University research groups also are involved in soil and plant studies.

14. TECHNICAL PROGRESS DURING FY 1972

Refer to schedule 189 for the following projects which were merged starting FY 1973: Plant Factors; Plant Physiological Ecology; Physiology of Mineral Accumulation in Plants; Soil Survey and Characterization; Quantitative Plant Ecology.

15. EXPECTED RESULTS IN FY 1973

Work will continue on ecosystem radionuclide and nutrient cycling studies. Emphasis will be placed upon plutonium cycling in collaboration with other problems coordinated under the Nevada Applied Ecology Group. Other radionuclides under continued investigation are the long-lived isotopes, Sr90 and Cs137, deposited in radioactive fallout areas downwind from the early, above-ground nuclear testing activities. Another survey will be conducted at the persistence study sites as part of our continuing 5-year interval study on ecosystem cycling of biologically significant radionuclides. Among those mineral nutrient elements involved in ecosystem cycling of which we are most interested at present are the macronutrients N, P, K, Na, Ca, and Mg and the micronutrients Fe, Mn, Cu, Zn and B.

Work will continue to study the influence of abiotic factors and nutrient gradients on plant characterization in the desert environments. Studies in cooperation with the US/IBP Desert Biome Program will include further work on patterning, association, and distribution of shrub species. Monitoring of environmental conditions at field study plots will continue. It is expected that several manuscripts will be developed from

results of computer analysis of certain aspects of soil-plant relationships in the desert ecosystem.

With the arrival of another principal investigator, more intensive work will be done on ecosystem cycling of carbon and water with emphasis on CO_2 exchange, decomposition, and seasonal retranslocation. In conjunction with this work, greater emphasis will be placed upon studies of root growth and studies of water balance and cycling. Daily and seasonal cycles of photosynthesis will be measured. Changes resulting from environmental manipulations, including that of ionizing radiation, will be studied. Transpiration rates will be measured simultaneously with each of the above conditions so that it will be possible to better understand water relations under conditions obtained in the field.

We shall continue studies on the recovery of disturbed environments, including artificial revegetation of areas having slow natural recovery. Some of this work will be done in conjunction with the plutonium cycling and redistribution studies. Work also shall continue on studies of the effects of environmental manipulations in the desert ecosystem. Additional tests of the effects of irrigation on ecosystem cycling of heavy metals will be initiated. It is hoped that FY 1973 will be a good year with respect to reproductive activity in perennial plants in order that further work can be done to assess the impact of long-term, low-dosage gamma radiation from the Rock Valley Cs137 source. Effects thus far have been manifest in reproductive tissues of the Ephedra nevadensis, but the past two years of sustained drought conditions have resulted in very poor and extremely erratic reproductive activity in most shrub species, regardless of location or exposure to ionizing radiation.

Field descriptions and chemical and physical characterization of soil from 79 profile sites have been completed. Characterization of the vegetation at these sites will be completed. All data shall be placed onto punch cards for computer analysis. Manuscripts shall be prepared on the soil characteristics and on the soil and plant interactions from these analyses.

All of the work undertaken by this team project is part of the total effort of the Environmental Radiation Division to develop models for a desert ecosystem.

16. EXPECTED RESULTS IN FY 1974

The work which has been projected for FY 1973 on studies encompassing ecosystem cycling of radionuclides and nutrient elements, including carbon and water, shall be continued and further amplified in FY 1974. It is anticipated that emphasis shall continue on the plutonium contamination problem and plans will be developed accordingly. Much of our work in FY 1974 shall involve more intensive ecosystems analysis with UCLA computer facilities using those data which have been acquired on the climatic, soil, and plant components of the desert environment during the past 5 to 7 years. Findings will be reported in research papers and descriptive monographs. We anticipate that by FY 1974 the work involving studies of plutonium dissemination in the environment shall require the help of another principal investigator, and this need has been projected for FY 1974.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: PLANT FACTORS

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
Evan M. Romney

8. Project Term:
From: 1953 To: 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	3	0	0
(b) Other Tech.	$\frac{1}{4}$	0	0
TOTAL:	$3\frac{1}{4}$	0	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 46,400	$ 0	$ 0
(b) Materials and Services	10,500	0	0
Sub-Total Direct Project Support	$ 56,900	$ 0	$ 0
(c) Indirect Expenses *	31,600	0	0
TOTALS:	$ 88,500	$ 0	$ 0

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

11. PUBLICATIONS DURING FY 1972

Effects of a Chronic Exposure to Gamma Radiation on the Shrub Ephedra nevadensis in the Northern Mojave Desert. Radiation Botany 11: 33-37, 1971 (with H. W. Kaaz and A. Wallace).

Some Interactions of Ca, Sr, and Ba in Plants. Agronomy Journal 63: 245-248, 1971 (with A. Wallace).

Radiation Doses to Vegetation from Close-In Fallout at Project Schooner. In: "Survival of Food Crops and Livestock in the Event of Nuclear War," D. W. Bensen and A. H. Sparrow (eds.), USAEC Office of Information Services, 1971, pp. 352-369 (with W. A. Rhoads, H. L. Ragsdale, and R. B. Platt).

Vulnerability of Crops to Fallout Beta Radiation. In: "Survival of Food Crops and Livestock in the Event of Nuclear War," D. W. Benson and A. H. Sparrow (eds.), USAEC Office of Information Services, 1971, pp. 603-632 (with other members of Committee 2).

Revegetation Problems Following Nuclear Testing Activities at the Nevada Test Site. In: "Radionuclides in Ecosystems," J. D. Nelson (ed.), 1972 (in press). (With A. Wallace and J. D. Childress).

Persistence of Radionuclides in Soil, Plants and Small Mammals in Areas Contaminated with Radioactive Fallout. In: "Radionuclides in Ecosystems," J. D. Nelson (ed.), 1972 (in press). (With W. A. Rhoads, A. Wallace and R. A. Wood).

Cycling of Stable Cs in a Desert Ecosystem. In: "Radionuclides in Ecosystems," J. D. Nelson (ed.), 1972 (in press). (With A. Wallace and R. A. Wood).

Ecological Aspects of Plutonium Dissemination in Terrestrial Environments. In: "The Biological Implications of the Transuranium Elements," W. J. Blair and R. C. Thompson (eds.), 1972 (in press). (With J. J. Davis).

Radioecology and Ecophysiology of Desert Plants at the Nevada Test Site, 1972. USAEC Monograph (in press), 450 pages (with A. Wallace).

12. SCOPE OF THE PROJECT

This research project will be merged with other related studies in FY 1973. Refer to Schedule 189 entitled, "Nutrient and Radionuclide Cycling," for the continuing Scope of the Project.

13. RELATIONSHIP TO OTHER PROJECTS

Refer to Schedule 189 entitled, "Nutrient and Radionuclide Cycling."

14. TECHNICAL PROGRESS DURING FY 1972

Work in cooperation with another section has continued in close-in fallout areas of three Plowshare nuclear excavation tests in order

to further investigate the biological cycling of radionuclides and further document the recovery and succession of vegetation. Two reports were made covering findings during the periods since fallout contamination was deposited at these sites.

Additional test plots were established in Yucca Flat and Frenchman Flat in support of work on artificial revegetation of disturbed sites with native desert species. Lack of natural revegetation at disturbed sites in the lower elevation basins of the Nevada Test Site during the past 20 years is convincing of the need for man's help in assuring revegetation of disturbed sites. A report was made on some problems of revegetation following nuclear testing activities at the Nevada Test Site.

Work continued to further investigate some ecological aspects of radionuclide dissemination in the desert environment. Emphasis was placed upon the biological cycling of plutonium in conjunction with studies under the coordination of the Nevada Applied Ecology Group (J. J. Davis, Nevada Operations Office). Two reports were made on this phase of our research program.

Some findings from collaborative laboratory studies at UCLA and field investigations at the Nevada Test Site, which have been in progress during the past five-year period, were summarized and assembled into a 450-page monograph. Much of the work included is of interest to and has direct application for the needs of the US/IBP Desert Biome program. We have cooperated in this program with work involving phenology and abiotic factor measurements, non-destructive dimensional analysis of vegetation for biomass estimation, primary productivity of major plant species, soils survey and characterization, and mineral element analysis of plant and animal tissues.

15. EXPECTED RESULTS IN FY 1973

See Schedule 189 entitled, "Nutrient and Radionuclide Cycling," with which this project has been merged.

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 AND ECOLOGY

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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: 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	1 1/2	0	0
(b) Other Tech.	1/4	0	0
TOTAL:	1 3/4	0	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 42,800	$ 0	$ 0
(b) Materials and Services	10,200	0	0
Sub-Total Direct Project Support	$ 53,000	$ 0	$ 0
(c) Indirect Expenses *	23,700	0	0
TOTALS:	$ 76,700	$ 0	$ 0

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

11. PUBLICATIONS DURING FY 1972

Wallace, A. and E. M. Romney. 1971. Some interactions of Ca, Sr, and Ba in plants. Agron. Jour. 63:245-248.

Wallace, Arthur. 1971. Effect of calcium levels on redistribution of Sr^{85} in bush bean plants. Plant and Soil 35:415-420.

Wallace, A. and Collaborators. (In press). Radioecology and Ecophysiology of Desert Plants at the Nevada Test Site. USAEC Monograph, 450 pp.

Wallace, A., V. Q. Hale, G. E. Kleinkopf, and R. C. Huffaker. (In press, Ecology). Carboxydismutase and phosphoenolpyruvate carboxylase activities from leaves of some plant species from the northern Mojave and southern Great Basin Deserts.

Wieland, P. A. T., Frolich, E. F., and A. Wallace. (In press, Madrono). Vegetative propagation of woody shrub species from the northern Mojave and southern Great Basin Deserts.

Romney, E. M., A. Wallace, and J. D. Childress. (In press, Proceedings of 3rd National Symposium on Radioecology, Oak Ridge,Tenn.) Revegetation problems following nuclear testing activities at the Nevada Test Site.

Wallace, A., E. M. Romney, and R. A. Wood. (In press, Proc. of 3rd National Symposium on Radioecology at Oak Ridge,Tenn.) Cycling of stable Cs in a desert ecosystem.

Romney, E. M., W. A. Rhoads, A. Wallace, and R. A. Wood. (In press, Proc. 3rd National Symposium on Radioecology in Oak Ridge, Tenn.) Persistence of radionuclides in soil, plants, and small mammals in areas contaminated with radioactive fallout.

Wallace, A., E. M. Romney, and A. M. Abou-Zamzam. (In press). Sodium relations in desert plants. I. Cation contents of some plant species growing in the Mojave and Great Basin Deserts and of some of the same species grown in a glasshouse.

Wallace, A. (In press, Health Physics) Increased uptake of ^{241}Am by plants caused by the chelating agent DTPA. (To be published June 1972)

12. SCOPE OF THE PROJECT

This project will be merged with related studies in FY 1973. Refer to Schedule 189 entitled, "Nutrient and Radionuclide Cycling" for the continuing scope of the project.

13. RELATION TO OTHER PROJECTS

See Schedule 189: "Nutrients and Radionuclide Cycling."

14. TECHNICAL PROGRESS DURING FY 1972

Physiological ecological studies were made at the Nevada Test Site of moisture conditions, of revegetation of denuded areas, of Cs cycling, of root growth and distribution, of recycling of nutrients within plants, of translocation of photosynthate into roots of nitrogen and phosphorus cycling in ecosystems, of phenological relationships, of interactions among desert vegetation, of lime and gypsum responses for vegetation, of salinity effects of tolerant and nontolerant plant species, of effects of water and salts on photosynthesis and transpiration in desert plants, and of gamma radiation effects on desert plants. Reports were made in appropriate publications concerning this work. The section gave some assistance to Nevada Operations Office on some plutonium studies at the Nevada Test Site. Effort was also expended in collecting environmental abiotic data for the International Biological Program, although the data are also useful for our own studies. The work of this section is cooperative with that of Plant Factors (Romney), Soil Characterization (Hale-acting) and Quantitative Plant Ecology (Bamberg - acting).

15. EXPECTED RESULTS IN FY 1973

See Schedule 189 entitled, "Nutrient and Radionuclide Cycling," with which this project has been merged.

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 - 1972

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: 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	1	0	0
(b) Other Tech.	0	0	0
TOTAL:	1	0	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 17,100	$ 0	$ 0
(b) Materials and Services	5,600	0	0
Sub-Total Direct Project Support	$ 22,700	$ 0	$ 0
(c) Indirect Expenses *	7,900	0	0
TOTALS:	$ 30,600	$ 0	$ 0

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

11. PUBLICATIONS DURING FY 1972

Wallace, A. and collaborators. (In press). Radioecology and Ecophysiology of Desert Plants at the Nevada Test Site. U.S.A.E.C. Monograph, 450 pp.

Al-Jibury, Laika K. Salt Tolerance of some desert shrubs in relation to their distribution in the southwestern deserts of North America. Ph.D. thesis, Arizona State University, 1972. (Much of work done at LNMRB under my guidance).

12. SCOPE OF THE PROJECT

This research project will be merged with other related studies in FY 1973. Refer to Schedule 189 entitled, "Nutrient and Radionuclide Cycling" for the continuing Scope of the Project.

13. RELATIONSHIP TO OTHER PROJECTS

Refer to Schedule 189 entitled, "Nutrient and Radionuclide Cycling".

14. TECHNICAL PROGRESS DURING FY 1972

Work has continued on the response of various Mojave Desert shrubs to water potentials reaching values of -60 to -75 bars. Plants are grown in containers (in some cases about 8 feet in depth) so that extent of the root system is known. Water potential is measured by the hygrometer method. Photosynthesis, respiration, and transpiration rates are measured as well as characteristics of recovery to added water. The preliminary data reported last year has been expanded and a manuscript is in preparation. While net photosynthesis exceeds respiration while water potentials are below -30 bars in many cases, root extension does not occur in soils where water potential is lower than a few bars.

Laboratory and field studies established that Atriplex canescens and A. polycarpa are more tolerant to high salinity levels than Artemisia tridentata and Larrea divaricata. Field studies of sites having either predominantly pure stands or mixed populations provided evidence that soil salt levels are a major edaphic factor affecting plant distributions in the desert.

Publications are being prepared from the thesis of Miss Al-Jibury. Additional work in this area is not contemplated in the immediate future.

15. EXPECTED RESULTS IN FY 1973

See Schedule 189 entitled, "Nutrient and Radionuclide Cycling" with which this project has been merged.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: QUANTITATIVE PLANT ECOLOGY

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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: 1971 To: 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2 ½	0	0
(b) Other Tech.	0	0	0
TOTAL:	2 ½	0	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 30,200	$ 0	$ 0
(b) Materials and Services	7,000	0	0
Sub-Total Direct Project Support	$ 37,200	$ 0	$ 0
(c) Indirect Expenses *	23,700	0	0
TOTALS:	$ 60,900	$ 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 1972

Bamberg, S. A. and H. N. Friesen. A precipitation estimation technique for developing isohyets in an arid area using vegetation and topographic parameters. Water Resources Bulletin. (Submitted)

12. SCOPE OF THE PROJECT

This project will merge with related studies in FY 1973. See the Schedule 189 on Nutrient and Radionuclide Cycling for the continuing scope of the project.

13. RELATIONSHIP TO OTHER PROJECTS

See Schedule 189 on Nutrient and Radionuclide Cycling.

14. TECHNICAL PROGRESS IN FY 1972

1. Analysis of the vegetational component of the IBP Desert Biome site in Rock Valley was completed. The site has been gridded on a 100 m coordinate system and divided into zones of relatively homogeneous vegetation on the basis of linear transects and interpretation of aerial photographs. On the site 190 randomly located linear plots were established and sampled for both annual and shrub plant parameters. Calculated for each zone and total site were plant cover, frequency, volumes and biomass reported by species and for each species by plant part. A field sampling procedure and computer program was developed for rapid analysis of vegetation on the desert. Most of this study was included in an annual report to the IBP Desert Biome.

2. Gaseous exchange measurements of CO_2 uptake and transpiration were completed for the 1971 field season in Rock Valley and at the glasshouse in Mercury. These results were summarized in the annual report to the IBP Desert Biome.

3. A preliminary model for relating CO_2 exchange to plant productivity utilizing the measurements of gaseous exchange was constructed. This model attempts to account for the discrepancy between the CO_2 fixed and the actual plant production.

15. EXPECTED RESULTS IN FY 1973

Refer to the Schedule 189 on Nutrient and Radionuclide Cycling for the continuing 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: SOIL SURVEY AND CHARACTERIZATION

3. AEC Budget Activity No.: 06 02 04

4. Date Prepared: April - 1972

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

6. Working Location:
UCLA and NEVADA TEST SITE

7. Person in Charge:
Verle Q. Hale

8. Project Term:
From: 1970 To: 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	$\frac{3}{4}$	0	0
(b) Other Tech.	$\frac{1}{4}$	0	0
TOTAL:	1	0	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 18,800	$ 0	$ 0
(b) Materials and Services	6,000	0	0
Sub-Total Direct Project Support	$ 24,800	$ 0	$ 0
(c) Indirect Expenses *	7,900	0	0
TOTALS:	$ 32,700	$ 0	$ 0

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

11. PUBLICATIONS DURING FY 1972

Listed in other sections.

12. SCOPE OF THE PROJECT

This project shall be merged with related studies in FY 1973. See the section on Nutrient and Radionuclide Cycling for the continuing scope of the project.

13. RELATIONSHIP TO OTHER PROJECTS

See the section on Nutrient and Radionuclide Cycling.

14. TECHNICAL PROGRESS IN FY 1972

Soil analyses (chemical and physical) were completed for 79 study sites. The data were placed onto computer cards so that correlations and regressions among variables can be determined. Radionuclide data were obtained for some of the profiles. Plant composition by emission spectrometry, neutron activation, and potentiometric titration were obtained for samples collected at the study site and similar calculations are made for the interactions among plant and soil characteristics. Manuscripts are being prepared on the soil characteristics, effects of plants on soil characteristics, radionuclide distribution in soils, available macro and micro element distribution in soils, and effects of soils on mineral composition of plants. These studies overlap with those of Plant Factors (Romney) and Physiological Ecology (Wallace).

15. EXPECTED RESULTS IN FY 1973

See the section on Nutrient and Radionuclide Cycling for continuing 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: MEDICAL PHYSICS INSTRUMENTATION

3. AEC Budget Activity No.:	4. Date Prepared:
06 02 08	April - 1972
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: 1972

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2	0	0
(b) Other Tech.	0	0	0
TOTAL:	2	0	0

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 25,000	$ 0	$ 0
(b) Materials and Services	8,000	0	0
Sub-Total Direct Project Support	$ 33,000	$ 0	$ 0
(c) Indirect Expenses *	13,000	0	0
TOTALS:	$ 46,000	$ 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 1972

None

12. SCOPE OF THE 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 associated methodology development and not in the direction of rapid or more convenient data handling and processing. Some of the new developments are being used in biological and medical research applications. Current efforts pertain to the development and application of the measurement of physical characteristics of viable cells and the use of physical methods to separate cells of different characteristics and ionizing radiation responses.

13. RELATIONSHIP TO OTHER PROJECTS

Dr. Marvin Van Dilla, Los Alamos Scientific Laboratory
Dr. Howard Mel, Donner Laboratory, University of California, Berkeley

14. TECHNICAL PROGRESS IN FY 1972

1. By the use of perfected density gradient and sizing techniques it has been found that after total body irradiation of rabbits there are significant changes in the density distributions of peripheral blood lymphocytes. Additionally, erythrocytes are prematurely ejected into the blood stream by the hematopoietic organs. The premature erythrocytes are about 50% larger in volume than the normal red cells but are not ordinary reticulocytes.

2. Basophils band in a region of the bovine serum albumin precision density gradient which region is free of neutrophils and eosinophils, but is contaminated with lymphocytes. Since basophils adhere to glass beads under certain conditions while lymphocytes do not, it should be possible to eliminate the lymphocyte contamination and obtain pure basophil preparations. Progress has been made to that end. Instead of making a glass bead column separation before making a density gradient separation the order is reversed. It has been shown that the density gradient procedure does not affect the viability or adherence of the cells in the glass bead column.

3. In cooperation with Dr. Esther Hays, a procedure has been developed for concentrating the colony forming cells of human and mouse bone marrow in specific fractions of density gradients which are used in subsequent colony forming assays. Control experiments show that three hour exposure of the cells to the density gradient medium (containing G-acid to prevent clumping) and subsequent recovery does not appreciably diminish the colony forming potential of cell samples.

Program discontinued as a result of the death of the Principal Investigator.

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 MEASUREMENTS PROGRAM

3. AEC Budget Activity No.: 06 02 08

4. Date Prepared: April - 1972

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

6. Working Location: UCLA

7. Person in Charge: Gerald C. Huth

8. Project Term: From: 1972 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	1/4	10	12
(b) Other Tech.	0	0	0
TOTAL:	1/4	10	12

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 4,000	$ 140,000	$ 182,000
(b) Materials and Services	19,000	68,500	73,200
Sub-Total Direct Project Support	$ 23,000	$ 208,500	$ 255,200
(c) Indirect Expenses *	2,000	76,500	94,800
TOTALS:	$ 25,000	$ 285,000	$ 350,000

* 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 1972

None

12. SCOPE OF THE PROJECT

This new program for the Laboratory of Nuclear Medicine and Radiation Biology has as its objectives the development of:

I A research group who will contribute to the science and art of radiation sensing and measurement. The disciplinary input required is drawn largely from areas of physics, particularly solid state physics, and engineering - especially electrical engineering.

II Applications utilizing radiation measurement technology and skill in:

a. Medicine and biology
b. Environmental Problems
c. Special problems with transuranium elements

Initial phases of the work are expected to be in the following areas:

1. Development of high atomic number crystal detectors

There is experimental evidence to support the potentiality of high "Z" detectors. Such a detector would have important applications to medicine particularly for positron emittor detection. Mercuric and lead iodide will be studied initially. A concerted effort will be undertaken to relate crystal characteristics, as measured by infrared spectroscopy, x-ray fluorescence, Hall effect, and detector performance, with crystal growth and purification methods. The character of the traps present in the materials will be studied with the aim of eliminating them. Useful detectors will be fabricated as soon as feasible and called for, although basic research will continue as a long range project.

2. High Purity Germanium Detector Applications

Work currently being carried out at Brookhaven National Laboratory shows that the successful exploitation of high purity germanium detectors in medicine (and also in environment measurements) will depend on the ability to assemble large arrays of detectors in order to obtain large volume and/or large area. At the same time noise performance must remain excellent, i.e., the array must retain an energy discrimination nearly as good as that of a single detector.

A high purity germanium detector of a size that can be fabricated with success from the presently available material (15 to 20 cm^3 volume) will be developed and presented in an encapsulated module, complete with its channel of electronics, from which any array or stack can be constructed.

Aims will be reliability, stability and simplicity of stacking and operating.

3. High Speed Detection Techniques

In ordinary solid state radiation, detector events are recorded quite fast - in tens of nanoseconds. In avalanche detectors processes are considerably faster. A project will be initiated to develop electronics to make use of the high speed inherent in detectors, clearly at the expense of energy resolution, although an effort will be made at keeping as much resolution as possible. A second aspect of the project will concern itself with the fast detection of small signals buried in noise by exploiting the different form of signals with respect to time in sub-nanosecond time domains.

4. Trapping in High Purity Germanium

The nature of traps existing in high purity germanium as grown or induced by irradiation will be studied. This basic research can ultimately help in obtaining better germanium from the crystal growers. Furthermore, an understanding of a "simple" material like germanium can open the way to a better understanding of other compound materials which are plagued by trapping effects, like CdTe and HgI_2.

Application Programs

a. Medicine

As indicated previously the development of high atomic number semiconductor detectors would have important implications for medicine. This is also expected to be true of some of the other projects delineated above. The underlying assumption in the strategy of a radiation measurement program in the sam laboratory with a strong medical research program is that the interaction between the groups will prove to be mutually beneficial. More specific description of application goals in medicine must await the establishment of radiation measurements program.

b. Environment

The comments in the previous paragraph apply also to applications to environmental or pollution problems. It seems clear that the capabilities of the radiation measurements group would be useful, for example, in studies of transport and fate of radionuclides from nuclear power plants or fossil fuel plants. The expertise of the group might also be useful in developing charged particle excitation procedures on the cyclotron. This capability would be potentially useful in various polution problems particularly atmospheric pollution. Interaction with other laboratory groups and colleagues from the campus is to be expected.

c. Transuranium Element Detection

The main objective of this effort is to implement techniques for significantly increasing the detection sensitivity for radioisotopes whose emanations result in a fluence of low energy photons. Uranium and transuranium isotopes fall into this class. The detection techniques employed will be based on silicon "avalanche" and other semiconductor radiation sensors conceivably arranged in arrays or

clusters. One point of emphasis for gaining increased sensitivity will be the reduction of the perceived background level. This effort will in essence, however, attempt to arrive at detection of nuclear interactions by their time signature, i,e., in the sub-nanosecond ($< 10^{-9}$ sec) time domain. To the extent that this becomes possible the effects of temperature, light, etc. on the nuclear radiation detection process are minimized. Applications would lie in extremely low level, in vivo counting of even lowest energy radiations.

13. RELATIONSHIP TO OTHER PROJECTS

The project relates to that of Dr. F. Goulding at Livermore. Other AEC supported programs at Battelle Northwest, Lovelace Foundation, Argonne National Laboratory relate to our proposed program. Interaction with Laboratory programs in Nuclear Medicine, Environmental Biology and Radiation Biology will develop.

14. TECHNICAL PROGRESS IN FY 1972

The first two members of this group joined the staff in June, 1972.

15. EXPECTED RESULTS IN FY 1973

This will be the first year of operation of the project. Initial effort will be the recruitment of staff. We expect to see the following progress made in basic areas:

1. It is expected that the germanium detector "module" described above will become operational and several other detectors for special applications will have been developed.

2. Some methods of crystal growth will have been examined and evaluated by the end of the FY 1972. It is expected that a good diagnosis of what element or imperfection is causing difficulties in detector characteristics will have been made.

3. Development of fast electronics with moderate energy resolution for high count rate applications will be underway. The theory of sub-nanosecond detection for very small signals should be in a good stage of development and some basic measurements should already be completed on the subject to provide guidance for the next steps.

4. Methods for measurements of trap characteristics should be well set up and operating. Characteristics of traps created by γ irradiation should be at a better stage of understanding that at present. A routine method of analysis of germanium ingot samples is expected to be completed.

Application research will depend on staffing progress and exploratory research and cannot be specified at this time.

16. EXPECTED RESULTS IN FY 1974

We expect continued progress in the basic research initiated in FY 73 and application research should be underway on medical and environmental problems.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: CLINICAL NUCLEAR MEDICINE

3. AEC Budget Activity No.: 06 03 01

4. Date Prepared: April - 1972

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

6. Working Location:
UCLA and HARBOR GENERAL HOSPITAL

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

8. Project Term:
From: 1958 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	5	6	6
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$
TOTAL:	5 $\frac{1}{4}$	6 $\frac{1}{4}$	6 $\frac{1}{4}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 61,500	$ 83,000	$ 84,000
(b) Materials and Services	17,200	18,500	19,100
Sub-Total Direct Project Support	$ 78,700	$ 101,500	$ 103,100
(c) Indirect Expenses *	47,900	48,600	43,700
TOTALS:	$ 126,600	$ 150,100	$ 146,800

* 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 1972

Poe, N.D. and Taplin, G.V.: Radioisotope Pulmonary Scanning. Nuclear Medicine, Chap. 12, pp. 322-349, W.H. Blahd (ed), McGraw-Hill, July 1971.

Isawa, T., Hayes, M., and Taplin, G.V.: Radioaerosol Inhalation Lung Scanning: Its Role in Suspected Pulmonary Embolism. J. Nucl. Med., 12, 606-609, Sept., 1971.

Isawa, T., Taplin, G.V., Beazell, J., and Criley, J.M.: Experimental Unilateral Pulmonary Artery Occlusion. Acute and Chronic Effects on Relative Inhalation and Perfusion. Radiology 102, 101-109, Jan. 1972.

Kitani, K. and Taplin, G.V.: Biliary Excretion of Tc-99m Albumin Microaggregate Degradation Products (A Method for Measuring Kupffer Cell Digestive Function?). J. Nucl. Med. (in press).

Kitani, K. and Taplin, G.V.: Catabolic Pathway Differences Between I-131 and Tc-99m Labeled Albumin Colloids and Microaggregates. J. Nucl. Med. (in press).

Hayes, M. and Moore, T.C.: Early Detection of Canine Renal Allograft Rejection by Reduction in the Scan Bladder/Kidney Isotope Intensity Ratio. Surgery, 71, 60-65, Jan. 1972.

Hayes, M. and Moore, T.C.: Early Detection and Classification of Renal Transplant Rejection by B/K Scan Ratio and Blood Isotope Clearance Data. Transplantation, 12, 139-141, Aug. 1971.

Hayes, M. and Maxwell, M.: The Renogram in Hypertension. Chapter in Radioisotopes in Kidney Disease. E. Blaufox (ed), (in press).

Hayes, M., Moore, T.C., and Taplin, G.V.: Radionuclide-Procedures in Predicting Early Renal Transplant Rejection. Radiology, 1972 (in press).

Kitani, K., Taplin, G.V., and Morinari, H.: Rapid Liver Accumulation of Human Serum Albumin in Sensitized Dogs. J. Nucl. Med., June, 1972 (abst.).

Hayes, M., Brosman, S., Taplin, G.V., Selin, C.E., and Terao, E.: Comparison of Ureteral Catheterization and Sequential Scanning Methods of Determining Individual Kidney Function. J. Nucl. Med., June, 1972 (abst.).

Taplin, G.V. and Robinson, G.D., Jr.: Radio-Rose Bengal-Albumin Microspheres. A Multipurpose Diagnostic Agent. J. Nucl. Med., June, 1972 (abst.).

Gates, G.F., Orme, H.W., and Dore, E.K.: Measurement of Cardiac Shunting with Technium Labeled Albumin Aggregates. J. Nuc. Med., 12, 746-749, Nov., 1971.

Taplin, G.V., Poe, N.D., Isawa, T., and Dore, E.K.: Comparison of Radioaerosol Versus Radioxenon Inhalation Procedures in Obstructive Lung Disease and Pulmonary Embolism. Chapter in the book Proceedings of the Symposium on Clinical Dynamic Function Studies with Radionuclides, Hahnemann Medical College, Phil., Pa., 1972 (in press).

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 five years 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 in operation since June 1, 1971. The laboratory's clinical research efforts will be continued at Harbor General Hospital at a somewhat reduced level for the next year or two 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 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 1972

Lung Function Studies (H. Morinari and G.V. Taplin): Lung scintigraphy studies in chronic obstructive airway disease, bronchogenic carcinoma and pulmonary embolism are continuing under Dr. Morinari at Harbor General Hospital who has replaced Dr. Isawa as of July 1971. Another continuing project is a study of lung reimplantation and transplantation in dogs - to determine whether or not lung denervation impairs ciliary activity and other lung clearance mechanisms. Preliminary results of two types of lung clearance studies indicate that the denervated lung removes 10-50μm size albumin macroaggregates deposited (via bronchial catheter) in the major bronchi in amounts and at rates similar to those in the opposite normal lung. The same statements are true for inhaled radioalbumin aerosol particles in respect to those deposited in the trachea and main airways. However, those reaching the lung periphery are retained for several hours at least.

Two other studies were initiated, each having to do with particle size of radioalbumin aerosols. The first was to devise means for the delivery of submicron sized radioalbumin aerosols. This project was initiated because submicron size aerosols should behave more like inert gases as indicators of air flow and penetrate to certain emphysematous lesions into which larger (1-5μ) aerosols fail to penetrate although they can be shown to have airway communication following radio xenon inhalation. Progress in this direction has been obtained by use of a heating chamber and/or by alterations in the length and caliber of the tubing and/or by placing additional baffles in the aerosol delivery line to the patient. These maneuvers have also improved the lung images following radioaerosol inhalation in specific respects. First, aerosol deposition in the trachea, major airways and stomach has been nearly eliminated in normal cooperative subjects and is far less of a problem in patients with airway disease. These improvements have not been accompanied by loss of capacity to detect airway abnormalities (as "hot" spots) which is an advantageous feature of aerosols for lung imaging.

Another related study is the attempted development of Radioaerosol Bronchography. (G.V. Taplin and K. Lyons): Such a procedure using relatively minute quantities of test material (1.0 mg albumin aggregates labeled with 0.5 to 1.0 mCi Tc-99m) could be employed without hazard to many patients in whom standard bronchography using grams of radiopaque materials is contraindicated. By the time of this writing (Febr. 25, 1972) numerous attempts to deliver 5-10μ microaggregates of albumin by inhalation as an aerosol have failed. Ultrasonic nebulizers reduce the size of the particles to 2.0μ or less during the 5-10 minutes of the inhalation procedure. Mechanical nebulizers (Bennett and Bird types) have the same effect and while giving excellent lung parenchyma images they do not increase large airway deposition as hoped and expected. Albumin microspheres (5-10μ) are not disrupted by high pressure or ultrasonic nebulization but these large particles fail to deposit on the trachea. They are captured in the delivery system by impaction and only the small particles reach the lungs - and these fail to deposit in sufficient quantities in large airways to permit visualization. Other methods and agents are under study.

Liver and RES Studies (K. Kitani, G.V. Taplin and H. Morinari): Rapid Liver Accumulation of Human Serum Albumin Sensitized Dogs. This investigation was undertaken to determine the fate of heterologous human serum albumin (HSA) solutions, when injected into dogs previously sensitized to this

commonly used radiopharmaceutical agent. However, either as colloidal size (10-20mμ) or 1-5μ microaggregate suspensions, HSA is rapidly removed from the blood in dogs and man (T-1/2=2-3 min.) while it accumulates in the phagocytic cells of the liver-spleen and bone marrow (RES organs). In previous (1965) toxicity studies in dogs to 10-80μ albumin macroaggregate suspensions, tracer doses (<0.1 mg/kg) were not sensitizing, but single large doses (20 mg/kg or more) nearly always produced immediate, severe anaphylactoid reactions when administered 7-10 days or longer after the sensitizing dose.

Following radio-HSA injections, blood samples were taken at frequent intervals for 3 hours and were assayed for radioactivity content. The heart and liver areas were monitored during the first 15 minutes. The distribution of radioactivity in the heart, liver, and spleen, was visualized sequentially by γ camera imaging.

In normal dogs, HSA remains in the circulation during the initial 3-hour period and the heart and liver areas are visible as blood pools only. Repeated 1.0 mg doses of HSA and single doses of 10 mg/kg failed to alter the blood disappearance rates of HSA or to produce observable reactions. However, in dogs given single 20 mg/kg or thrice repeated 10 mg/kg doses, both the I-131 and Tc-99m HSA solutions were rapidly removed from the blood and found in the liver at 15 minutes by camera imaging. At 3 hours, Tc-99m had entered the gallbladder as previously found and reported with Tc-99m albumin microaggregate suspensions. For control purposes, in similarly (HSA) sensitized dogs, the fate of canine serum albumin I-131 was not altered and no reactions were observed, in contrast to the anaphylactoid type reactions encountered in sensitized dogs.

In conclusion, the rapid accumulation of labeled HSA solutions in the liver of dogs previously sensitized to heterologous HSA is most probably caused by their immediate conversion in the blood to particulate antigen-antibody (albumin-globulin) complexes which are removed by the RES organs by phagocytosis, as are other particulates, such as carbon particles, bacteria, and viruses. The catabolic pathway of this albumin-globulin complex following initial Kuppfer cell trapping, appears to be quite similar to that of Tc-99m albumin microaggregates, which are also initially engulfed by the RE cells degraded to smaller molecular size, transferred to the hepatic cells and then excreted in the bile. Finally, the grossly altered behavior and metabolic fate of HSA solutions when injected into sensitized dogs is of both practical and scientific interest. It could give rise to serious pitfalls in animal studies with this agent, but more importantly, these findings warrant further investigation and confirmation at the cellular level through immunologic and autoradiographic studies now in the planning stage.

Liver-Spleen Scanning and Kupffer Cell Digestive Function Studies: Using a newly developed multipurpose diagnostic radiopharmaceutical agent (first described in the Progress Book under Radiodiagnostic Agent Development), studies in dogs have revealed the rapid initial deposition of microspheres in the RE cells of the liver and spleen, with subsequent excretion of the I-131 rose bengal label via the biliary tract beginning 40-60 minutes post injection. This organ distribution is in marked contrast with the slower uptake of I-131 rose bengal alone, which is limited exclusively to the polygonal cells of the liver with excretion via the biliary tract beginning 10-20 minutes post injection.

Potentially this agent should permit more accurate and quantitative assessment of Kupffer cell digestive function clinically, because the rose bengal

label is excreted exclusively by the hepatic cells. Also, it provides a means for demonstrating at the cellular level, by autoradiography, that the I-125 rose bengal in albumin microspheres initially engulfed by the RE cells is subsequently transferred to the hepatic cells and excreted in the bile. Clinically, the new agent may have special value as a dual purpose liver-spleen scanning agent for the detection of space occupying lesions and for subsequent sequential imaging of the biliary system in jaundiced patients as an aid in distinguishing intra- from extra-hepatic causes of biliary tract obstruction.

Metastatic Liver Disease - Correlative Studies (G.V. Taplin, K. Lyons, R. Gray and E. Terao): A collaborative project has been initiated both at the UCLA Center for the Health Sciences and at Harbor General Hospital - to study various liver lesions by three separate procedures namely; I.V. liver scans with Tc-99m sulphur colloids, hepatic arteriography followed by liver scanning after injection of Tc-99m albumin macroaggregates (MAA) in the catheterized hepatic artery.

These three procedures complement each other and when used in selected patients wherein such information is vital to proper management, they are hoped and expected to improve diagnostic accuracy and to aid in subsequent treatment. Once the patient has been catheterized and studied angiographically, the added scan following arterial MAA injection adds no significant risk while providing extremely useful information regarding the distribution of the liver's arterial blood supply on a regional basis. Such information is of particular value in locating small areas of increased arterial flow not detectable by either routine scanning or hepatic angiography. The triple technique should definitely aid the diagnosis of both primary and metastatic liver tumors because they are almost exclusively supplied by the hepatic artery. Such tumors should give findings distinctly different from cysts and abcesses which are generally devoid of blood supply, either arterial or portal venous. Initial clinical results appear most promising and projects at both hospitals are underway.

Kidney Function Studies (M. Hayes, T.C. Moore, and G.V. Taplin): The kidney transplant studies at Harbor General Hospital during fiscal year 1972 have been presented at national meetings and described in two publications and in another paper now in press in the J. Radiology (1972). The latter is entitled "Radionuclide Procedure in Pridicting Early Renal Transplant Rejection".

Radionuclide procedures generally are more sensitive and responsive indicators of sudden changes in kidney function and blood flow than are serum chemistry levels, which require time to reach new equilibrium states. Furthermore, the radionuclide tests, particularly the sequential kidney-bladder imaging procedure, provide evidence for or against intra and post renal obstruction, information which is not given by standard tests of kidney function.

Finally, in the management of kidney transplant recipients, radionuclide procedures, especially the B/K ratio, derived from the sequential kidney-bladder camera imaging procedure, are considered to be more sensitive and reliable than other methods for obtaining objective evidence of transplant dysfunction in the pre-rejection period.

15. EXPECTED RESULTS IN FY 1973

Lung Function Studies: Work in chronic obstructive lung disease and pulmonary embolism is to be continued. Further effort is to be placed on devising a practical method of radioaerosol inhalation bronchography. Related studies on evaluating the relative sensitivity of aerosol scanning vs. certain tests of lung ventilatory capacity are to be continued with special emphasis on studying patients with early bronchitis and asthma of mild degrees. In addition, chronic obstructive pulmonary disease patients will be studied repeatedly to determine if one can distinguish reversible from irreversible localized airway disease by serial scanning at regular intervals such as every 2-3 months for several years.

It has been demonstrated that dogs, previously sensitized to Human Serum Albumin (HSA) have anaphylactoid reactions when given relatively minute challenge dose of HSA I.V. Such reactions are associated with rapid accumulation of HSA particles in the RES organs. Inhalation studies will be made using HSA aerosols to learn more of the nature of respiratory tract allergy and possible abnormal distribution of inhaled HSA antigen.

Liver and RES Studies: It is hoped that certain liver and RES studies, conducted during the past several years, will be reactivated upon obtaining the funds and finding a suitable replacement for Dr. Kitani. Should we be successful, numerous studies of both scientific and potential practical clinical value are now in the planning stage. Most of these are related to exploiting the newly developed rose bengal I-131-HSA microspheres for testing Kupffer cell digestive function as an indicator of nonspecific immunity levels in diseases known to involve the RES, particularly, Hodgkins and other lymphomatous disease.

Metastatic Liver Disease Study: This project is expected to be continued in the Center for the Health Sciences and also at Harbor General and Long Beach Memorial Hospitals in an attempt to improve the diagnostic capabilities of standard radiocolloid liver scans and hepatic angiography by the addition of macroaggregate (MAA) liver scanning via the hepatic arterial route following angiography. It is likely that other organs such as the kidney and pancreas may be investigated in similar fashion.

Liver Autoradiography (M.L. Griswold and G.V. Taplin): Such studies will be made in conjunction with the animal and clinical investigation of rose bengal I-131-HSA microspheres in an attempt to demonstrate their initial entrapment by the RE cells of the liver and spleen and their subsequent digestion and transport to the liver's polygonal cells.

Kidney Function Studies (M. Hayes, T.C. Moore and G.V. Taplin): Collaborative studies in renal transplantation will be continued at Harbor General Hospital with Dr. Moore in further investigation of the rejection phenomenon. In addition to the clinical work, studies in animals are to be conducted to learn more about pathophysiological changes during the few days prior to the onset of gross signs of rejection. Such work will involve autoradiographic examination of the kidneys at the cellular level with Hippurate I-125. The latter studies will be performed in the Nuclear Medicine Research Laboratory at the Center for the Health Sciences by M.L. Griswold.

16. EXPECTED RESULTS IN FY 1974

During this year it is anticipated that adequate supplies (100 mCi lots) of I-123 will become available from either Brookhaven or Los Alamos. In this event, significant improvements will be made possible regarding currently used diagnostic radionuclides such as, in albumin micro and macro-aggregate suspensions, in hippuran and rose bengal, for kidney and liver imaging and for direct use in thyroid function studies. With most of these I-125 labeled agents, relatively little basic investigation will be needed but clinical studies are necessary to demonstrate the advantages, to establish new routines and to ferret out unsuspected disadvantages or side effects.

The clinical research section likewise anticipates the development of new radionuclides from the cyclotron and the radiopharmaceutical development sections. Special hopes are placed on improved bone and pancreas scanning agents.

Regardless of these anticipated innovations, work will continue in the areas of lung, liver-spleen-bone marrow imaging, RES and kidney function and in the problems of autoimmunity with kidney and lung transplantation.

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	3	3 1/4	3 1/4
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	3 1/4	3 1/2	3 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 38,100	$ 44,000	$ 49,000
(b) Materials and Services	11,400	13,900	14,900
Sub-Total Direct Project Support	$ 49,500	$ 57,900	$ 63,900
(c) Indirect Expenses *	24,700	25,900	24,900
TOTALS:	$ 74,200	$ 83,800	$ 88,800

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

11. PUBLICATIONS DURING FY 1972

Poe, N.D.: The Effects of Intracoronary Arterial Injection of Radioactive Albumin Macroaggregates on Myocardial Hemodynamics and Function. J. Nucl. Med., 12, 724-731 (1971).

Poe, N.D.: Comparative Myocardial Uptake and Clearance Characteristics of Potassium and Cesium. J. Nucl. Med. (in press).

Poe, N.D. (intro. by G.V. Taplin): Postural Influence on Lobar Distribution of Blood Flow in Dogs. Proc. Soc. Exp. Biol. Med. (in press).

Poe, N.D. and Taplin, G.V.: Radioisotope Pulmonary Scanning. Nuclear Medicine, W.H. Blahd (ed.), McGraw-Hill, Chap. 12, pp. 322-349, July (1971).

Poe, N.D.: Effects of Glucose-Insulin-Potassium Infusion on the Intramyocardial Distribution of Potassium/Cesium-131 in Experimental Infarction. J. Nucl. Med., 12, 386 (1971) (abst.).

Poe, N.D.: Comparison of Radioactive Potassium and Cesium for Myocardial Scanning. Clin. Res. 20, 209 (1972) (abst.).

Poe, N.D.: Soluble versus Particulate Radiopharmaceuticals for Regional Coronary Flow Determinations. Clin. Res. 20, 209 (1972) (abst.).

12. SCOPE OF THE PROJECT

The primary goal of this section is to study and/or develop new radionuclide techniques under experimental conditions. Our function not only is to determine the safety, limitations, and practical applications of specific procedures, but also to use them for basic physiologic and pathophysiologic investigations. In addition, the technical facilities of our laboratory are made available to other investigators in the UCLA Medical Center in either collaborative efforts or as a service function. For the latter, reciprocation is expected in the form of technical or professional services or supplies.

For several years the major efforts of this section were directed towards pulmonary research. In the past year activities have been primarily redirected to myocardial investigations. This change was due to the institution of cyclotron operations and a NIH contract obtained conjointly with the Division of Cardiology for development of noninvasive methods for evaluating myocardial infarction. This project is designed for the orderly investigation of radionuclides which localize in the heart. Most of those of immediate interest are cyclotron produced. Using the scintillation camera with computerized data analysis, attempts will be made to localize and quantitate regions of ischemia. Both animal and human studies are underway. Animal models are prepared to simulate the ischemic and infarcted changes anticipated clinically. To date, only cesium-129 has been used in patients but potassium-43 and long chain fatty acids labeled with carbon-11 should be available for use in the near future.

Lung scanning after the administration of radioaerosols continues to be a worthwhile field of investigation in spite of slow clinical acceptance for evaluation of bronchopulmonary diseases. It also has a potential place in air pollution research although this particular area has not yet been explored. Our immediate interest is in determining alveolar deposition and

clearance of wet aerosol preparations by autoradiography. Clinical studies employing long term serial scanning in chronic lung disease with and without therapy is planned at the affiliated Sepulveda Veterans Hospital.

13. RELATIONSHIP TO OTHER PROJECTS

Other sections in this division are exploring applications or radioaerosol scanning under the direction of Dr. G.V. Taplin. Myocardial investigations of similar nature to our work are being carried out by a number of investigators: Kriss at Stanford, Budinger and Yano at the Donner Laboratory, Ashburn at San Diego, Wagner et al. at Johns Hopkins.

14. TECHNICAL PROGRESS IN FY 1972

a. Cesium and Potassium Kinetics in the Myocardium. Cesium-129 and potassium-43 can be cyclotron produced and are usable as myocardial scan agents. Both have similar scanning energies. A number of dog experiments were carried out to determine uptake and clearance characteristics of these two tracers. Following intravenous injection, cesium gradually reaches a peak in the heart between 1 and 3 hours, and clears slowly with a half time of approximately one day. Potassium rapidly accumulates in the heart clearing with a half time near six hours. About 5% of the injected dose of each radionuclide is extracted by the heart. Following intracoronary arterial injection 22% of cesium and 72% of potassium is initially extracted. Clearance half times are approximately 6 and 1 hours respectively.

b. Cesium Versus Radioactive Particle Uptake After Intracoronary Injection. Radioactive particles have been used to determine regional myocardial blood flow following direct coronary arterial injection. The potential hazard of excessive arterial blockade with particles lead to the investigation of soluble cesium as a substitute. In dogs several different particle suspensions labeled with technetium-99m were injected simultaneously with cesium-131. Three models were used. First, injections were made into the left atrium in normal dogs. Second, left atrial injections were made after ligation of the anterior descending coronary artery. Third, direct injections were made into the anterior descending coronary artery. In the first group similar scan results were obtained by either method. Direct tissue counts showed even distribution of cesium throughout the myocardium but considerable irregularity in distribution of the particles particularly the larger particles. After coronary artery ligation, scan defects with technetium were discretely outlined. The defects with cesium were less well defined and considerable cesium was detected in the ischemic area. The distribution patterns following direct coronary artery injection were also dissimilar. The technetium particles were limited to a smaller and more clearly defined area although sometimes very obvious irregularities in the distribution patterns were evident by in vitro scanning and by direct tissue counts. The primary distribution pattern of cesium was similar, though slightly larger than the technetium pattern but again large amounts of cesium were found throughout the myocardium. The less well defined uptake of cesium both with direct injection and after coronary artery ligation is attributed to poor myocardial extraction, anastomotic channels, and recirculation. For clinical investigation it was concluded that cesium by direct injection would probably give similar results to those obtained by the particles but in a much safer manner.

c. Clinical Investigations with Cesium-129. Eighteen patients have had scans performed after intravenous and/or intracoronary arterial injections

of cesium-129. These patients were all undergoing cardiac catheterization for coronary artery disease or other cardiac abnormalities. The uptake of cesium following intravenous injection was so poor in relation to the overlying skeletal muscles that satisfactory scans could only be obtained with rectilinear scanners in the anterior projection. However, with intracoronary injections, anterior and right and left oblique images could be obtained quite satisfactorily with a scintillation camera using a pinhole collimator. However, delineation of small defects was rather poor. It is anticipated that our results will improve with the availability of computer analysis and background subtraction. Target-to-background ratios following intravenous injection were at best 4 to 1 and following intracoronary injection 7 to 1. Our preliminary conclusion is that cesium-129 by intravenous injection will not be a satisfactory means for detecting myocardial ischemia. Further investigations are planned using potassium-43 and carbon-11 labeled fatty acids.

d. Autoradiography. Attempts to determine alveolar distribution of inhaled radioaerosols (such as albumin-125) to date have not been successful. To remove any possibility of disturbing the deposited aerosol, frozen section technqiues are being attempted. However, it is extremely difficult to satisfactorily quick freeze and section the very friable inflated lung tissue, but further attempts in this regard are in progress.

e. Collaborative Studies. To determine the minimum pulmonary embolic lesion that can be detected either angiographically or by scintiscanning, in vivo clots were prepared in a number of dog preparations and were released into the lungs. Small, moderate, and large amounts of clot preparations were made following release of these clots. Scans and angiograms were performed and the animals sacrificed. The distribution and size of the clot and the diameter of vessels in which they were impacted were determined at autopsy. Although neither the scan nor the angiogram identified all the lesions, both techniques picked up at least some of the defects in each of the animals. Correlation between the scans and the angiograms were excellent based on interpretation made by independent observers. (Work performed in conjunction with Dr. Stephen Levy, Department of Medicine.)

f. Research Service to Other Investigators. Two groups outside the nuclear medicine division have been carrying on long range investigations using our facilities. The first is a project by the Department of Surgery in which they are using isotopes to study ventilation and perfusion changes in transplanted lungs in dogs. The second is a project by several members of the Department of Radiology who are using renography to study renal changes in dogs with radiated kidneys. Radiation is given to the animals while one kidney is being infused intra-arterially with epinephrine. This is done to protect the kidney from the harmful effects of high oxygen concentrations during radiation. It is being employed clinically in an attempt to maintain renal function after pelvic irradiation for carcinoma of the ovary.

15. EXPECTED RESULTS FOR FY 1973

The major effort in the coming fiscal year will be devoted to improving our techniques for myocardial scanning. We anticipate that our results will be improved in two areas. The first will be instrumentation. An on line computer attached to our scintillation camera will provide a means of background subtraction, contrast enhancement and statistical smoothing of myocardial scans. Data from multiple regions of interest will be available and will enable us to obtain quantitative values in areas of presumed

ischemia. This system will also permit stop motion studies in maximum diastole which will not only reduce motion artifact but will also stop the heart in its least contracted state. Improved radiopharmaceuticals will be the second approach used to improve myocardial scanning. It is quite likely that potassium-43 will possess no unique advantage over cesium-129. However, fatty acids have been shown to localize in the heart with a relative high specificity and these acids do not concentrate in the overlying skeletal muscle. The high energy of carbon-11 makes good resolution difficult and the very short half life further compounds the difficulties of imaging with this isotope. This problem will be partially overcome by using fluorinated fatty acids initially and later it is expected that certain fatty acids can be iodinated with I-123. The I-123 label would provide a far superior radiopharmaceutical.

The computer will also provide very high framing rates which can be used to determine numerous data points throughout the cardial cycle. From these points, curves can be drawn and values important to the cardiologists, such as systolic ejection fraction etc. can be determined. By using high concentrations of very short-lived isotopes, for example, red cells labeled with oxygen-15 carbon monoxide, very high count rates should be obtained without giving excessive radiation to the patients.

Specific experimental models will be developed to determine the uptake and clearance characteristics of cesium, potassium and fatty acids at various stages and degrees of pulmonary ischemia and infarction. It is known that potassium is lost from dead or dying cells. It will be important to determine just how rapidly this loss occurs and what is the relationship of the degree of loss to the degree of ischemia. Ischemic lesions will be prepared by surgical ligation of different coronary vessels. Animals will be sacrificed at varying times up to durations of six months after ligation. Prior to sacrifice the isotope under investigation will be injected simultaneously into the left atrium with small technetium labeled microspheres. The latter will serve as the true blood flow reference.

The frozen section technique for quick freezing lung tissue following aerosol inhalation will be expanded with assistance from the Department of Pathology. Not only will an attempt to determine the initial aveolar distribution of the agents that most likely will be used for aerosol scanning but also an attempt will be made to determine by similar means the clearance mechanism of these particles. It is also hoped that some preliminary investigations using indium-111 can be made. This radionuclide has a suitable energy for scanning and a half life of sufficient length for the performance of 24 hour delayed scans without giving excessive irradiation to the patient. This material is producible by our cyclotron although to date the amounts prepared have been too small for adequate aerosol scanning. I-123 albumin may prove to be even a better radiopharmaceutical for this purpose, if we are able to obtain this latter radionuclide in sufficient quantity.

16. EXPECTED RESULTS IN FY 1974

The developments for this fiscal year will be a direct extension of previous years activities. Major interest will continue in myocardial imaging and quantitation of regional ischemia. Work previously done in the research and catheterization laboratories will be extended to the coronary care unit for application to patients with acute myocardial infarctions. One of the fatty acid compounds labeled with I-123 will probably provide a suitable radiopharmaceutical for this purpose. Acquisition of additional funding,

presumably from outside sources, will be mandatory for expansion in this direction, however.

With the experience, animal models, technical expertise and equipment acquired over the preceding 2-3 years, we will maintain an active program for development and evaluation of myocardial specific radiopharmaceuticals. Nuclear Medicine almost certainly offers the greatest potential of any approach for noninvasive techniques for evaluation of the myocardium. Progress in this area will be slow but consistent and it seems quite likely that a separate investigational section comprised of a research physician, biochemist-radiopharmacist and technician should be established to expand this work.

Efforts will continue to experimentally establish the value of radioaerosol inhalation scanning and encourage its clinical application. This goal will be accomplished by continued basic work in our laboratory and short and long-term clinical studies in patients with chronic lung diseases at the affiliated Sepulveda Veterans Administration Hospital. Particular emphasis will be placed on determining the effects of various therapeutic regimens on the inhalation scan image pattern and the consequent use of the scan image in classifying and predicating the responses. Outside assistance will be required for the clinical activities.

This section will remain open to new ideas and potential investigational efforts in other areas. However, because of the heavy commitment to the previously outlined courses of action, these efforts will be primarily collaborative rather than independent.

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 - 1972

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 1972	FY 1973	FY 1974
(a) Scientific	2 1/4	2	2
(b) Other Tech.	1/4	1/4	1/4
TOTAL:	2 1/2	2 1/4	2 1/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 30,600	$ 29,000	$ 30,000
(b) Materials and Services	7,700	6,900	7,000
Sub-Total Direct Project Support	$ 38,300	$ 35,900	$ 37,000
(c) Indirect Expenses *	24,700	17,000	15,100
TOTALS:	$ 63,000	$ 52,900	$ 52,100

* 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 1972

Levy, J., Barnett, E.V., MacDonald, N.S., Klinenberg, J.R., and Pearson, C.M. Decreased Immune Globulin G and M Synthesis on Azathioprine Therapy. J. Clin. Invest. (in press)

Lucas, H.F., Jr., Markun, F., May, H., MacDonald, N.S., and Sweeney, J.: Thorium Daughters in the Spleen of a Thorotrast Case. Health Physics (Submitted 11/71).

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 1972

A total of 74 studies of the metabolic turnover rates of serum immune proteins (immune globulins, G and M) were made in patients suffering from rheumatic disorders. This work was conducted in collaboration with Dr. Joshua Levy and others of the Department of Medicine. The program is aimed at elucidating some of the factors in rheumatic diseases and providing methods for objective evaluation of the efficacy of therapeutic measures. Our contribution consisted in measuring the turnover rates of the two proteins (which have been labeled 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 from total body counting were combined with other information regarding serum concentrations, plasma volume, and body weight to calculate the daily rate of catabolism and synthesis of these vital proteins. In a series of 18 patients with active rheumatoid arthritis it was found that 4 months of treatment with the immunosuppressive drug, "Azathioprine," reduced the rate of synthesis of immune globulin-M by 41% (± 8% S.D.) of their pre-treatment synthetic rate (in mg. per kg. per day). After an interim period during which a placebo was administered regularly, the synthetic rate was found in general to be somewhat higher than the pre-Azathioprine base line level. Six of these patients were then placed on Cyclophosphamide therapy, after which the IgM synthetic rate was found to have been reduced dramatically (61% ± 11 reduction)! Immune globulin-G synthetic rates were also depressed by the drugs, but not to as great an extent. Cyclophosphamide decreased the synthesis of IgG more than azathioprine, however. In another series consisting of untreated patients and normal subjects, it appeared that the synthetic rate for Immune globulin-M in 8 patients with psoriatic arthritis was similar to that in 7 normals (9.1 ± 4 mg/kg/day compared to the normal 6.0 ± 1), whereas 26 rheumatoid arthritis patients were making IgM (and catabolizing it) at a mean rate of 21.7 ± 6 mg/kg/day!

The Total Body Counter (TBC) was also used to determine the amounts of gamma radioactivity in a number of adult residents of Southern California. Body burdens of Cesium-137 (which enters human food supplies as "fall-out" of the debris from atmospheric nuclear detonations) have been monitored continuously at UCLA since 1959. During calendar 1971 the mean quarterly values for Cs-137 contamination in males was 23 picocuries per gm. of body potassium, and 20 pCi/gmK for females. A total of 135 measurements were made. These levels are about the same as for calendar 1970 and suggest that human body contamination with Cs-137 has leveled off following the rapid decline of 1965-1968. Since atmospheric fall-out is declining, this may possibly imply that some Cs-137 is re-cycling and thus helping to establish a steady state condition. An alternative explanation might be that the observed level of Cs-137 represents a quantity of the nuclide which is more permanently fixed in the body than amounts which exceed this level. (Cs-137 in amounts above the nanocurie/gmK range appears to be metabolized in the body and excreted with the most important exponential "half-times" being between 1-3 months.)

Studies of the oral absorption and body retention of iron were continued, in collaboration with Dr. Wm. Figueroa of the Dept. of Medicine. Nine patients with hemochromatosis were given oral doses containing both Fe-59 and Cobalt-57. The percent absorption and retention was determined for each nuclide by repeated total body counts over a period of 3-4 weeks, together with serial blood sampling. The intent is to discover whether or not derangements of cobalt metabolism accompany the abnormal iron metabolism in this disease.

15. EXPECTED RESULTS IN FY 1973

A significant number of new radioactive agents intended for diagnostic uses in nuclear medicine will be developed by other workers in the Laboratory and Medical Center during this period. These will have to be tested in animals to evaluate their toxicity, tissue distribution, routes and rates of excretion, and other features of their metabolic behavior. Examples include cyclotron-produced Carbon-11 labeled compounds, Ba-135m and Ru-97.

The Total Body Counter will continue to be used in collaborative research on the metabolism of labeled immune serum globulins and other proteins; and in clinical metabolic studies with radionuclides of iron, cobalt and possibly

copper and zinc. Monitoring of members of the local population for Cs-137, and examination of persons exposed to radioactive materials in their work (radiochemists, cyclotron and reactor personnel) will continue.

16. EXPECTED RESULTS IN FY 1974

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.: 06 03 01

4. Date Prepared: April - 1972

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

6. Working Location: UCLA

7. Person in Charge: Gerald D. Robinson

8. Project Term: From: 1967 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	3 1/2	3 1/2	5 1/2
(b) Other Tech.	0	0	1/4
TOTAL:	3 1/2	3 1/2	5 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 50,000	$ 50,000	$ 78,000
(b) Materials and Services	12,500	14,500	15,700
Sub-Total Direct Project Support	$ 62,500	$ 64,500	$ 93,700
(c) Indirect Expenses *	29,500	29,100	40,300
TOTALS:	$ 92,000	$ 93,600	$ 134,000

* 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 1972

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 preparations 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 1972

Particulate agents (5-50μ), although initially developed nearly a decade ago, continue to be used in ever expanding variety of applications. Of special interest with some of the newer hemodynamic and/or perfusion scanning techniques are particulate radiopharmaceuticals with relatively narrow size ranges. A system which employs sedimentation from homogeneous suspension to fractionate particles into narrow size ranges has been developed. The particulate sample is brought into suspension and then is allowed to settle for an appropriate time interval. A given fraction of the uppermost portion of the settling suspension is removed, the removed volume is replaced with pure solvent and the process is repeated. Following several cycles at the initial settling interval essentially all of the particles smaller than some precise size limit have been removed from the system. The remaining particles are removed fractionally by repeated cycling at progressively reduced settling intervals. This is a universally applicable system which can be adapted to any type of particle/solvent system, with nearly any degree of precision in the size of individual particle fractions.

This system has been used to size microfine ion exchange resins (1-50μ) in aqueous suspension. Fifteen cycles (25 percent removal) at each of 15,10,5 and 2 minute settling intervals gave particles in the ranges 1-10, 10-15, 15-20, 20-30 and 30-50μ, respectively (95% within the specific range). This technique is being applied to the sizing of aqueous suspensions of human serum albumin (HSA) macroaggregates and HSA microspheres.

The participation of small ions or molecules in specific metabolic processes is potentially a powerful mechanism for improvements in the localization of radiopharmaceuticals in target organs. A program has been initiated to develop gamma emitting analogs of some biologically active molecules by utilizing some of the short-lived isotopes produced with the Biomedical [illegible]lotron.

Potassium fluoride in a polar, aprotic solvent is commonly used to prepare a variety of fluoroorganic compounds from the corresponding, bromo, chloro or iodoorganics. This interhalogen exchange reaction has been adapted to prepare a series of F-18 fluorocarboxylic acids. Initial work (with F-18 fluoroacetate) involved a 2-4 hr. reaction at 200°C between dry KF-18 and ethyl chloroacetate in acetanide or diethylene glycol solvent. The product was isolated by gas chromatography. Recently the technique has been modified by utilizing F-18 fluoride absorbed on anion exchange resins as a dry reagent for exchange (150°C; 1 hr.) with ethyl bromocarboxylates (no solvent required). As before, the product is isolated by gas chromatography. The biological behaviors of the F-18 fluorocarboxylic acids are being evaluated with particular attention to their abilities to follow the metabolic routes of normal carboxylic acids.

Micro-particulate radiopharmaceuticals (1-5μ) are rapidly removed from the circulation by the reticuloendothelial system (RES). 1-5μ size I-131-rose bengal-human serum albumin (HSA) microspheres have been prepared by heating a stirred homogenized suspension of I-131-rose bengal-HSA complex in cottonseed oil at 125°C for 1 hr. Studies in dogs have demonstrated the rapid initial deposition of the microspheres in the RE cells of the liver and spleen, with subsequent excretion of the I-131-rose bengal label via the biliary tract beginning 40-60 minutes post injection. This is in marked contrast with the slower uptake of I-131 rose bengal alone, which is extracted exclusively by the polygonal cells of the liver with excretion via the biliary tract beginning 10-20 minutes post injection.

A brief attempt to prepare and evaluate the complexes of Tc-99m (IV) and In-113m (III) with disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) as potential bone seeking radiodiagnostic agents has met with minimal success. Analysis by thin-layer chromatography (TLC) indicated that less than 25% of reduced $^{99m}TcO_4$ (ascorbic acid alone and with $FeCl_3$) was bound to EHDP. Direct complexation of In-113m (III) with EHDP has been somewhat more promising (in some case > 90% binding), but a reliable preparative routine has not yet been developed.

15. EXPECTED RESULTS IN FY 1973

In addition to continued work on several specific short term projects (i.e., rose bengal-HSA microspheres, ethane-1-hydroxy-1,1-diphosphonate complexes, etc.), emphasis will be placed upon expanded efforts to begin developing a few continuing programs which involve some of the more basic aspects of radiodiagnostic agent development. These would include:

1) Extension of the F-18 labeling program to include other likely biologically active compounds (steroids, purines and pyrinidines, amino acids, etc.); in addition, Br or I for methyl substitutions will be investigated as a possible second route to analog compounds.

2) A comprehensive study of the preparation and labeling of various types of particulate radiodiagnostic agents will be initiated. Special emphasis will be placed upon developing improved techniques for post preparative labeling of such agents. An automated version of our particle sizing system will be constructed and evaluated.

3) Application of gas chromatographic techniques to the labeling and processing of radioactive agents will be investigated. Potential applications include: carrier-free separation of labeling reaction products; processing of raw target materials from the Biomedical Cyclotron; heterogeneous (gas, solid) exchange labeling on gas chromatographic columns.

16. EXPECTED RESULTS IN FY 1974

Continued emphasis will be placed upon particulate agent development. The gamma emitting analog work will be extended to include the "shorter" lived cyclotron produced isotopes (i.e., N-13, C-11, O-15 - these would not represent "analog compounds", but the true physiologic molecules). Application of "modern" chemical techniques (gas chromatography, polarography, etc.) will be continued. An investigation into the chemical state of Tc-99m in molecules of biomedical interest will be initiated.

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE Field Office	BIOLOGY AND MEDICINE Program

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

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

2. Project Title: CLINICAL STUDIES: SHORT-LIVED ISOTOPES

3. AEC Budget Activity No.: 06 03 01

4. Date Prepared: April - 1972

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

6. Working Location: UCLA

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

8. Project Term: From: 1967 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2	2	2
(b) Other Tech.	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{1}{2}$
TOTAL:	2 $\frac{1}{4}$	2 $\frac{1}{2}$	2 $\frac{1}{2}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 24,600	$ 26,000	$ 27,000
(b) Materials and Services	12,500	13,100	13,200
Sub-Total Direct Project Support	$ 37,100	$ 39,100	$ 40,200
(c) Indirect Expenses *	15,800	15,400	14,200
TOTALS:	$ 52,900	$ 54,500	$ 54,400

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

11. PUBLICATIONS DURING FY 1972

Webber, Milo M., Victery, Winona, and Cragin, Michael D., "Demonstration of Thrombophlebitis and Endothelial Damage by Scintiscanning," Radiology 100:93-97, July 1971.

Webber, Milo M., Cragin, Michael D., and Victery, Winona, "Pulmonary Scanning using Technetium-99m Labeled Macroaggregates of Albumin Prepared According to a New and Simplified Technique," Am. J. Roent., Rad. Ther. and Nucl. Med. 113:4:690-692, December 1971.

Tetalman, Marc R., Hoffer, Paul B., Heck, Larry L., Kunzmann, Axel, Gottschalk, Alexander, Webber, Milo M., Resnick, Lawrence, Cragin, Michael, and Victery, Winona, "Appearance of the Lung Scan in Normal Individuals," J. Nucl. Med. 12:6:401, June 1971. (abstract)

Resnick, L., Webber, Milo M., Victery, Winona K., and Cragin, Michael D., "Blood Clot Localization Studies in 'Normal Subjects'," J. Nucl. Med. 12:6:458, June 1971. (abstract)

Webber, Milo M., Victery, Winona, and Cragin, Michael D., "Experimental Demonstration of Thrombophlebitis and Endothelial Damage," J. Nucl. Med. 12:6:471-472, June 1971. (abstract)

Cragin, Michael D., Webber, Milo M., and Victery, Winona K., "Effect of Aluminum Concentration in Technetium Eluent on Particle Size," J. Nucl. Med. 12:6:476, June 1971. (abstract)

Webber, Milo M., Cragin, Michael D., and Victery, Winona, "Atropine as a Possible Agent to Prevent Xerastomia in Patients Treated with ^{131}I for Carcinoma of Thyroid," J. Nucl. Med. 12:6:482-483, June 1971. (abstract)

Webber, Milo M., Cragin, Michael D., and Victery, Winona K., "Aluminum Content in Eluents from Commercial Technetium Generators," Letter to the Editor, J. Nucl. Med. 12:10:700, October 1971.

Webber, M. M., and Goodrich, J., "The Effect of Aluminum on Technetium Sulfur Colloid," Am. J. Roent., Rad. Ther. and Nucl. Med. 113:4:791-792, December 1971 (abstract of exhibit). (abstract)

Bosnjakovic, Vladimir, Bennett, Leslie R., Vincent, William, and Larson, Jeanne, "Dual-Isotope Method for the Determination of Intracardiac Shunts," J. Nucl. Med. 12:6:341-342, June 1971. (abstract)

Bosnjakovic, Vladimir, Bennett, Leslie, and Vincent, William, "Diagnosis of Intracardiac Shunts without Cardiac Catheterization," Circulation, Supplement II, Vol. XLIV, No. 4, abstract No. 535, p. II-144, October 1971.

Mowat, Paul, Lupu, Andrei, and Maxwell Morton, "Limitations of the Xe^{133} Washout Technique in the Estimation of Renal Blood Flow," Am. J. Physiol. (in press).

Parker, James D., and Webber, Milo M., "The Use of Iodine-131 Labeled Antibody in the Detection of Coccidioidomycosis," Society of Nuclear Medicine Abstracts, Third Annual North/South Meeting, September 1971, San Diego, California.

Surprenant, Edgar L., Wilson, Archie, and Bennett, L. Robert, "Clinical Application of Regional Pulmonary Function Studies," Radiology 99:3:623-631, 1971.

Parker, James D., and Bennett, Leslie R., "Effect of Water Ingestion on Spleen Size as Determined by Radioisotope Scans," Acta Radiologica 11: 4:385-392, July 1971.

Small, Richard C., and Bennett, Leslie R., "Normal 67-Ga Scan," J. Nucl. Med. 12:394, 1971. **(abstract)**

12. SCOPE OF THE PROJECT

It is the objective of this project to expand the clinical application of short-lived radioisotopes, both those commercially available and the cyclotron-produced radionuclides, by the development and refinement of new radiopharmaceuticals and by the study of their clinical applications in the detection and diagnosis of disease. Programs instituted under this project have ranged from the determination of criteria for defining a normal lung scan, to the development of a new technique for assessing vascularity of the femoral head, to a study of the value of such isotopes as gallium-67 and indium-111 in the demonstration of soft tissue disease.

Radionuclide particle preparations of less and greater than capillary size (such as thiosulfate produced technetium-99m sulfur colloid, originally developed in this project) have found wide clinical use in defining lung disease and liver abnormalities and in the detection of occult blood clots. Current studies analyze new techniques, such as radioisotope angiography for the diagnosis of intracardiac shunts, which offers the advantage of avoiding cardiac catheterization, and the use of pertechnetate and technetium labeled albumin in the early detection of degenerative arthritis, which may prove important to the beginning of a program of preventive medicine.

Radioisotopes produced by the biomedical cyclotron will be evaluated and tested in clinical applications. For example, cyclotron-produced radioactive gases may become useful in the evaluation of lung function.

13. RELATIONSHIP TO OTHER PROJECTS

This project is related to the radiodiagnostic agent development sections and to those of the basic nuclear medicine sections and biomedical cyclotron facility. In addition, this research is closely coordinated with the practice of nuclear medicine in the University Hospital Nuclear Medicine Clinic. Experimental patient studies with the nuclides discussed earlier are considered part of the experimental design after extensive animal experimentation has shown safety and effectiveness of the radionuclide.

The labeled fibrinogen project mentioned is related to USPHS grant project entitled "Radioscintigraphic Demonstration of Thrombophlebitis," No. 5-R01-GM-17113 and is considered complementary to it. In addition, trainees and fellows supported by an N.I.H. training grant have participated in some of these projects.

14. TECHNICAL PROGRESS IN 1972

Demonstration of Vascularity of the Femoral Head using Technetium Sulfur Colloid: The purpose of this study is to determine the feasibility of demonstrating the vascularity of the femoral head using technetium sulfur colloid. Hip disease including fractures of the femoral neck raises questions relating to the blood supply of the femoral head. An index of such vascularity may prove valuable in demonstrating the prognosis of the healing of a fracture of the femoral neck and could be very helpful as a guide to the orthopedic surgeon in determining if immobilization, pinning or prosthetic replacement of the femoral head is necessary.

The method used in this study depends upon the ability of phagocytic cells located within the bone marrow to concentrate particles of ^{99m}Tc-sulfur colloid. These particles are normally used for liver and spleen scans. However, sufficient accumulation within the bone marrow is commonly noted to outline the bony structure of the pelvis. The method used in this study consists of intravenous administration of such radiocolloid in doses commonly used for liver scans, studying the area of the pelvis including the femoral heads by photoscan technique, and correlating the scans obtained with roentgenograms taken in the same position by an overhead x-ray tube. The procedure has been performed over a period of two years in patients who were scanned primarily for question of liver disease. These patients were examined to obtain information on the normal appearance of distribution of phagocytic activity within the bone marrow of the pelvis. In essentially all instances, symmetry of uptake was noted. Some patients demonstrated very excellent uptake of tracer within the femoral head, but symmetry was characteristic of normal patients. Patients with known hip disease were also studied; asymmetry was noted in such patients. In cases where the femoral head was surgically absent and replaced with a prosthesis, no uptake in the area could be seen. In cases of degenerative joint disease, asymmetry was noted with increased uptake in the involved joints. Studies on patients with acute fractures of the femoral neck are currently in progress.

Concentration of technetium sulfur colloid in bone marrow can be elevated by momentarily increasing marrow circulation during the injection of the tracer colloid. This can be accomplished by treating the extremity with microwave diathermy before injection of the tracer. Studies in rabbits have shown a 40% increased concentration of tracer in treated tibiae over non-treated. A similar effect can be accomplished with epinephrine which apparently gives bone marrow circulatory preference, possibly as a result of vasoconstriction of the splanchnic circulation. Although epinephrine has shown an average of 63% increase in concentration of the tracer over the control group, its side effects may be undesirable.

In conclusion, there is value in using colloidal material as an index of blood flow to the femoral head. Uptake is symmetrical in patients with no known hip disease. Asymmetry of uptake in the region of the femoral head correlates with a disease process of the femoral head.

Detection of Subclinical Arthritis with Technetium Pertechnetate or RISA: Early detection of arthritis is important in the prevention of irreversible joint damage. Increased vascularity of joints, an antecedent of clinical arthritis, can be detected with pertechnetate-99m or RISA. Preliminary studies on rats with induced rheumatoid arthritis have shown that the extent of experimental arthritis can be estimated by the concentration of tracer in the joint. This information may be clinically useful in delineating a program of preventive drug therapy. This study is ongoing and is expected to continue into FY 1973.

Radiocolloid Lymphangiography: Radiocolloidal gold-198 is the popular tracer for lymphatic system visualization; however, the long half-life and high energy beta ray emission of gold-198 gives undesired tissue irradiation. Two radioisotopes, indium-111 and technetium-99m sulfur colloid, were evaluated as suitable replacements due to their relatively short half-lives and low beta emissions. Lymphatic system visualization in rabbits and dogs using these two radionuclides was comparable to scintiscans done with gold-198. In addition there is the advantage of reducing irradiation to the surrounding tissue. Because of large particle size leading to slow movement and its short half-life, Tc-S appears less desirable than ^{111}In colloid in these preliminary studies.

The Use of Iodine-131 Labeled Antibodies in the Detection of Coccidioidomycosis: A diagnostic technique for differentiating the pulmonary form of chronic systemic fungal diseases from pulmonary tuberculosis would be useful. One possible technique is the administration of radioisotope labeled antibodies which might localize in areas of fungal infection and which could be detected in vivo with scintiscanning equipment. In this study, mice with localized subcutaneous Coccidioides immitis infection were given one of the three following I-131 labeled rabbit globulin preparations: (1) globulin from a normal rabbit, (2) globulin from a C. immitis infected rabbit containing precipitins to the fungal antigen, or (3) a non-IgG fraction of preparation #2 containing C. immitis precipitins. In

each of the three experiments two mice were sacrificed 3, 24, 48, and 96 hours after injection and the amount of radioactivity was determined in the coccidioidal lesions and in several tissues. Also, at 96 hours scintiphotos were obtained of the lesions of two mice in each experiment using the Anger scintillation camera with a pinhole collimator. The mice given preparations #2 or #3 tended to have higher lesion-to-tissue radioactivity ratios than those given preparation #1, and their scintiphotos tended to show the lesions more clearly. The main problems encountered in the study were high body background due to prolonged circulation of the labeled globulin, and nonspecific protein localization in the coccidioidal lesion. Future work will require antibody purification and labeling with higher specific activity as well as reduction of body background.

Radioisotope Angiography: A radioisotope angiographic method for the diagnosis of intracardiac shunts has been developed and is under investigation. Visualization of flow through the heart is made with a scintillation camera placed in a modified left anterior oblique position with information stored in a tape system for later replay and analysis. Two radioactive tracers are injected intravenously in rapid sequence: xenon-133 in saline to outline all intracardiac venous blood flow exclusive of the pulmonary venous return, and technetium-99m as the sulfur colloid to visualize all chambers. Time-activity curves obtained from regions-of-interest selected over the heart chambers are obtained during the replay of the tape. Comparison of curves from the two isotopes makes it possible to identify chambers and the approximate magnitude and location of shunts. The method also appears to be capable of detecting the existence of shunts at the atrial or ventricular level.

Indium-111 EDTA Cisternography:

Indium-111 has been termed the ideal isotope for cisternography by many researchers. This is due to its high photon yield, low energy gammas, lack of beta radiation, and suitable half-life. The use of In-111 labeled transferrin for cisternography has been reported by others. The present study was designed to show that In-111 EDTA would give high quality scans, also. It was expected that the whole body dose would be less than either the popular I-131 HSA or In-111 Transferrin since EDTA would be rapidly excreted by the kidney once the complex was absorbed from the cerebrospinal canal. A second part of this study involved quantitative measurements over the head and trunk to estimate a biological half-life. Last, the study was to show that In-111 EDTA in the cerebrospinal canal would be safe for human use.

Indium-111 chloride was complexed with Calcium Disodium Versenate. The pH was adjusted to 7.4 with NaOH and the preparation was autoclaved. A dose of 250 uCi was injected into the cisterna magna of 5 dogs, the spinal arachnoid space being too small for easy injection. Scintiphotos were obtained with a gamma camera at 2, 6, 24, 48, and 72 hours, while quantitative measurements were made with a 2-inch scintillation crystal at the same time, with the

addition of a count at injection time and at one hour. The crystal was placed 12 inches from the head and 12 inches from the trunk with a lead shield at the neck separating the two areas. The urine was collected and counted to determine the percent dose excreted. Also, cerebrospinal fluid (CSF) samples taken prior to injection and seven days later were analyzed for cells and protein.

The indium-111 EDTA gave a particularly high ratio of CSF activity to body background, yielding high quality scintiphotos. The scintillation probe counting yielded a biological half-life of about 12 hours and the urine data were in general agreement. For animals in which the indium-111 EDTA was suspected to have leaked from the subarachnoid space into the circulatory system (extensive bleeding through the spinal needle) the biological half-life was appropriately shorter. The urine data, corrected for decay, showed that 80-90% of the indium-111 was recovered in the urine by six days post injection. This further substantiates the claim of whole body absorbed dose reduction. The laboratory analysis of CSF showed no evidence of an inflammatory reaction to the In-111 EDTA.

It is concluded that In-111 EDTA gives high quality scintiphotos and is safe for human use. Quantitative studies using a scintillation probe are useful in estimating turnover rates of the isotope, and are expected to be of diagnostic value when used in humans with interthecal injection. Likewise, urine counting will add some quantitation to cisternography.

Appearance of the Lung Scan in Normal Individuals: The theoretical "normal" has been referred to in many texts and articles dealing with lung scanning, although no broad study of normal individuals has defined the limits of variation of the scan appearance in populations with no evidence of pulmonary or other disease. This presentation describes the appearance of lung scans in a group of normal subjects.

The population consisted of 42 volunteers between the ages of 21 and 34. Thirty were males, 12 were females. All were nonsmokers. Injections were made in the same manner as the previous study using macroaggregates of albumin labeled with ^{99m}Tc whose size ranged from 10 to 70 microns with an average of 50 microns. The scan dose was approximately 3 mc. All images were made on an Ohio Nuclear dual 5-inch scanner using low energy (24L) collimators. Four views were made on all subjects.

Six of the 42 subjects (14%) exhibited perfusion defects. These abnormalities included two crescent defects, two apical perfusion defects, one wedge defect and one basilar defect. Two of these six subjects had major scan abnormalities indistinguishable from pulmonary emboli. The abnormalities in the other four subjects were subtle. The scans in an additional four subjects demonstrated other abnormalities including two with "hyperexpanded" lungs and two with loss of the normal perfusion gradiant.

Normal ^{67}Ga Scan: Gallium-67 has been shown to have a high uptake in a variety of soft tissue tumors. It also has a high uptake in liver, spleen, and bone, as well as excretion through the bowel which can present problems in interpretation. Based on 60 cases with total body scans, we have attempted to define the characteristics of a normal gallium-67 scan by analyzing our findings in areas of the body free of cancer.

In scans of the head, there is always some uptake in the region of the mouth; whether this is in the soft tissues or bone has not yet been determined. The amount of Gallium retained in this area after 24 hours is usually quite low; and since its distribution takes on a symmetrical pattern, it has not interfered with tumor localization. A similarly low level symmetrically distributed uptake also occurs in the posterior portions of the skull, corresponding to the marrow-containing regions. The neck normally shows a small amount of radioactivity in the cervical vertebrae and occasionally in the soft tissues of the anterior neck.

In the thoracic area, there is a low but definite uptake in the ribs and scapulae on posterior scans. Again, this is not a problem since lesions in the ribs and vertebrae are usually much hotter than surrounding tissue. On anterior scans of the chest, the sternum often presents a more difficult problem in interpretation because it normally has a relatively high uptake; consequently, there is a very real chance of missing tumors in or just below the sternum. Techniques for scanning this area still need improvement, and greater experience will be required to establish criteria for accurate diagnosis. The shoulders, which are usually scanned with the chest, have a moderate uptake, but here the ability to compare activity between the two sides makes interpretation quite easy.

The upper abdomen probably presents the greatest problems in interpretation. In the normal scan there is a high uptake in the liver and spleen as well as in the lumbar vertebrae and pelvic bones. Excretion by way of the bowel and urinary tract may also cause problems, particularly at 24 and 48 hours. It is obvious that laxatives and enemas will be necessary, probably beginning at 24 hours and that no reliable information is gained by scanning before 48 hours.

An understanding of the normal pattern of distribution between the liver, spleen, and bone is essential if lesions involving these organs are to be consistently diagnosed. Also, careful standardization of the radiopharmaceutical preparation of the gallium-citrate complex will be required to insure accurate control of pH and the prevention of colloid formation.

15. EXPECTED RESULTS IN FY 1973

A cooperative effort with the orthopedic service will be made to evaluate the clinical usefulness of technetium sulfur colloid as a scanning agent for visualizing the femoral head and neck. An extensive program of animal and patient studies will be initiated to determine the reliability of

pertechnetate as an early detector of degenerative arthritis. Based on previous animal work, indium-111 EDTA for cisternography will be clinically evaluated with the cooperation of the UCLA Neurology Clinic. Indium-111 cisternography can be used to establish the extent and degree of spinal canal traumatic injury and is useful in documenting the results of therapy. Subcutaneously injected colloidal indium-111 for lymphatic system visualization will be compared to radiographic lymphangiography to establish its usefulness. Whole body scanning using gallium-67 and indium-111 will demonstrate whether or not these nuclides may be useful in routine demonstration of sites of Hodgkin's disease and staging of tumors. Fatty acids labeled with cyclotron-produced carbon-11 will be studied in the demonstration of suspected cardiac insufficiency. If available, carbon-11 labeled amino acids may provide an improved means of evaluating the morphology and function of the pancreas.

16. EXPECTED RESULTS IN FY 1974

Clinical applications of extremely short-lived cyclotron-produced radionuclides such as oxygen-15, nitrogen-13, and carbon-11 will become possible if the installation of a high speed product transfer tube linking the cyclotron with the associated Nuclear Medicine Clinic is accomplished by this time. Microspheres or macroaggregates labeled with such tracers will be evaluated as clot localization agents. Large doses can be given with very little radiation exposure. Better images and greater radiation safety are expected.

Evaluation of the thrombogenic effect of certain routinely taken drugs such as contraceptives will be studied with allied clinics, using radiolabeled fibrinogen and particulate clot localizing agents. Post-operative patients will also be included in this study. It is hoped that the presence of thrombosis may be determined before embolization can take place. Evaluation of the nature of the scintillation photo and renogram studies of the kidney in acute tubular necrosis (ATN) in experimental animals is proposed. ATN is increasingly seen as a diagnosis in the transplanted kidney as well as in the patient who is post-operative and has suffered renal failure. It is expected that a four-way correlation of histology, function, scintiphoto appearance, with time of anoxia may help clarify the significance of the renogram and sequential kidney images in ATN.

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.:	4. Date Prepared:
06 03 01	April - 1972

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

7. Person in Charge:	8. Project Term:
Norman S. MacDonald	From: 1971 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	4	5 $\frac{1}{2}$	6 $\frac{1}{2}$
(b) Other Tech.	1 $\frac{1}{2}$	1 $\frac{1}{4}$	1 $\frac{1}{4}$
TOTAL:	5 $\frac{1}{2}$	6 $\frac{3}{4}$	7 $\frac{3}{4}$

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 88,700	$ 98,000	$ 111,000
(b) Materials and Services	29,000	50,500	57,300
Sub-Total Direct Project Support	$ 117,700	$ 148,500	$ 168,300
(c) Indirect Expenses *	57,400	56,700	57,100
TOTALS:	$ 175,100	$ 205,200	$ 225,400

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

11. PUBLICATIONS DURING FY 1972

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 1972

The final acceptance tests of the accelerator were completed satisfactorily and the facility began productive operation on 12 July 1971. Mechanical performance has been excellent and "down-time" due to malfunctions or breakdown has been remarkably low. For example, during the period from 12 July 1971 to 1 March 1972, a total of 183 individual bombardments were performed in 159 normal working days. The accompanying table summarizes the production of radionuclides during this period. It should be noted that for each of these nuclides special techniques had to be devised and tested, not only for holding the target, but also for isolating the desired radionuclide after the bombardment. A number of novel "targetry" devices and appliances were designed and constructed, and several new chemical extraction procedures were developed for separations of the radionuclide mixtures.

The radionuclide which was made most frequently was Cesium-129. We found that this could be obtained in moderate yields by alpha bombardment of purified sodium iodide powder pressed into a 1" diam. depression in an aluminum target plate. When necessary, isolation of pure Cs-129 completely free of iodine and Na-24,22 could be achieved by extraction of the aqueous solution with tetra-phenyl boron in benzene, and recovery from the organic phase by reextraction with HCL and neutralization.

Identification was made by gamma spectroscopy with a Ge-Li detector and 1600 channel analyser, together with half-life measurements. After demonstration of the sterility and apyrogenicity of Cs-129 prepared in this way, the nuclide was approved for human use.

A simple technique for preparing Potassium-43 was devised. Argon gas is pumped into a gold-plated, hollow brass cylinder 8" long to 8-15 psig. This is bombarded, end-on, through an AL foil with alphas. The carrier-free K-43 is then simply washed out of the tube with physiologic saline and sterilized. The value of Cs-129 and K-43 for myocardial imaging in patients is under active investigation by Dr. N.D. Poe at the UCLA Medical Center.

Indium-111 has been reported to offer advantages for cisternal scanning and for localization of certain tumors. It is being evaluated by Dr. L.R. Bennett and his group in the UCLA Medical Center. We studied two different routes to this nuclide; bombardment of natural Cadmium with deuterons, and natural Silver with alphas. In-111 from a Ag foil target was less contaminated with other short-lived radionuclides of In and was free of the trace of long-lived In-114m, all of which appear in the product from Cd. A liquid-liquid extraction method, using a heptane solution of diethyl-hexyl-phosphate was developed for the rapid, simple isolation of the radio-indium from the dissolved target metal. The In-111 is prepared routinely once or twice a week, put into the appropriate pharmaceutical form and delivered to Dr. Bennett's group for administration to patients on an investigational basis.

Considerable effort was devoted to the preparation of several aliphatic monocarboxylic acids, labeled with Carbon-11. This radionuclide was readily obtained by proton bombardment of a flowing stream of nitrogen gas containing a trace of oxygen. Carrier-free carbon-11 monoxide and carbon-11 dioxide are produced. The CO was converted to CO_2 by passing the gases over hot copper oxide. The radioactive CO_2 was then bubbled into a stirred ethereal solution of the appropriate Grignard reagent. The reaction mixture was then hydrolyzed with HCl and the resulting carboxylic acid was extracted with NaOH or $(NH_4)_2CO_3$. Thus, for example, C-11-acetate was made from methyl magnesium bromide; C-11-octanoate from n-heptyl magnesium bromide; and C-11-palmitate from pentadecyl magnesium bromide. These acids were not isolated, but were used as aqueous solutions of the sodium salts for tissue distribution and organ imaging studies in animals. Although C-11 has only a 20 minute half-life, these chemical manipulations were rapid enough to make activities in the range of 5-20 millicuries available for injection. Preliminary studies of these preparations in dogs by Dr. Poe's group suggested they had little or no value for heart imaging, probably because the C-11 label was on the terminal carboxyl group which is subject to rapid metabolic degradation. However, there is a possibility that deviations from the normal rapid rate of metabolic degradation of such compounds may be observed in various diseases and functional disorders. Accurate recording of the count rate data obtained within the first minute or two following injection would provide the raw information for kinetic analysis to demonstrate such deviations.

Nitrogen-13, in the form of carrier-free, N-13-ammonia was prepared by deuteron bombardment of either a flowing stream of methane, or of a layer of aluminum carbide powder. This was used by Drs. Cohen and Spolter of the Sepulveda Veterans' Administration Hospital to prepare N-13 labeled glutamine by an enzymatic reaction. The yields of radioactive glutamine were quite low due to the 10 minute half-life of the N-13. However, the experiments were significant because of the practical knowledge gained in a promising field - the rapid and specific labeling of compounds with radionuclides by the use of enzymes fixed on a solid medium.

A number of other radionuclides were made in relatively small amounts for exploratory purposes: Fluorine-18 for preliminary experiments in labeling acetate by exchanging F-18 for bromine atoms in ethyl bromoacetate (Dr. G.D. Robinson); direct labeling of oleic acid with energetic F-18 atoms produced by deuteron bombardment of neon gas; Ruthenium-97 (for evaluation as a general labeling nuclide) by alpha bombardment of natural molybdenum; Barium-135m from the bombardment of cesium chloride with helium-3 (for evaluation as a bone-scanning agent); and Chlorine-34 from the (He-3, alpha) reaction on KCL (for studies of electrolyte movements in extravascular fluid spaces).

Other activities included: the bombardment of soil samples with alpha particles to study the dose dependence of production of thermoluminescent centers (Dr. Nishita); the production of radionuclides for labeling insects to permit tracking studies (Dr. Edney); and preliminary experiments on the analysis of N-15 by charged particle activation.

15. EXPECTED RESULTS IN FY 1973

It is anticipated that the useage of Cs-129 will decline, so that only occasional runs will be required. Requirements for K-43 will remain at about the present level through mid-year. There are indications that the demands for In-111 to explore fully its potentialities for tumor localization and for cisternal scanning will amount to three times the present production, at the least. Therefore, means for increasing yields of this nuclide will be sought. One approach would be to bombard the target with the internal, rather than with the external beam, thus doubling the maximum intensity of particles available at the target. This would entail the design and construction (or purchase) of a special probe and vacuum system for internal irradiations. Work on the labeling of various organic molecules with F-18 will be intensified, in close conjunction with the Radiopharmaceutical Development Section. In particular, reactions involving exchange of F-18 with non-radioactive halogen atoms in the compounds will be investigated. Also, attempts will be continued to achieve direct labeling of molecules with "hot" F-18 atoms whose kinetic energies have been moderated down to the epithermal energy range. Gas-liquid column chromatography techniques will be utilized as a means of separating the components of the reaction mixtures. Attention will be given to preparing Iodine-123 of suitable purity and in sufficient quantity to label various compounds in 2-20 millicurie lots. I-123 labeled human serum albumin, rose bengal, and hippuran will be among the first to be prepared for clinical evaluation.

Work with Carbon-11 will concentrate on preparing labeled amino acids, and possibly some simple carbohydrates. Ruthenium-97 and Ba-135m will be produced in quantities sufficient for evaluation of their potential as radiopharmaceutical agents. Investigation of the potentialities of activation analyses with charged particle beams will continue.

16. EXPECTED RESULTS IN FY 1974

During this period most of the nuclides mentioned above will become routinely available in quantities to satisfy the clinical research requests. We may expect that a number of "new" radionuclides will also be suggested for investigation. Further exploitation of the biomedical potentialities of Carbon-11 will depend on the development of rapid methods of carbon labeling and purifying organic compounds of physiologic significance.

It is hoped that the technology of conducting enzyme catalysed reactions on solid media (columns, thin surface coatings, etc.) will have developed sufficiently to provide applications to Carbon-11 labeling problems. Interest in clinical applications of Oxygen-15 (2 minute half-life) and Nitrogen-13 (10 min.) will probably develop sufficiently so that the logistical problems of transporting these nuclides from the cyclotron to the nuclear medicine clinic will become critical. Hopefully, installation of a pneumatic tube delivery system would solve this difficulty.

RADIONUCLIDE PRODUCTION (12 JULY 1971 - 1 MARCH 1972)

Nuclide	Target	Reaction	No. of Runs	Use
^{129}Cs	NaI	α,2n	50	Myocardial imaging (patients & animals)
^{111}In	Cd or Ag	d,n or α,2n	41	Tumor localization; cisternal scans (patients & animals)
^{43}K	Ar	α,P	5	Myocardial imaging (animals)
^{11}C	N_2	P,α	9	^{11}C-acetate
			6	^{11}C-octanoate
			7	^{11}C-palmitate
			2	^{11}C-monoxide - red cell labeling
^{13}N	CH_4 or Al_4C_3	d,n	18	Enzymatic labeling - glutamine
^{18}F	H_2O	^3He,P	9	Exchange labeling exps. (F-18-acetate)
^{18}F	Ne	d,α	3	Hot atom labeling exps. (F-18-oleate)
^{97}Ru	Mo	α,2n	11	Evaluation as a general labeling agent
^{123}I	Te	d,n	1	Evaluation of purity for labeling purposes
^{135m}Ba	CsCL	^3He,P	2	Potential bone scanning agent
^{34}Cl	KCL	^3He,α	1	Exploratory
^{56}Co	Fe	P,n	2	Gamma energy calibration standard

Evaluation for imaging heart and other organs (animals)

SCHEDULE 189

ADDITIONAL EXPLANATION FOR OPERATING COSTS
RESEARCH AND DEVELOPMENT ACTIVITIES

SAN FRANCISCO OPERATIONS OFFICE
Field Office

BIOLOGY AND MEDICINE
Program

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

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

2. Project Title: LEUKEMIA BIOLOGY

3. AEC Budget Activity No.: 06 03 01

4. Date Prepared: April - 1972

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, Ph.D.

8. Project Term:
From: 1955 To: Continuing

9. Man Years:	FY 1972	FY 1973	FY 1974
(a) Scientific	2 3/4	1 3/4	1 3/4
(b) Other Tech.	1	1	1
TOTAL:	3 3/4	2 3/4	2 3/4

10. Costs:	FY 1972	FY 1973	FY 1974
(a) Direct Salaries	$ 46,600	$ 38,000	$ 38,000
(b) Materials and Services	12,000	10,100	10,200
Sub-Total Direct Project Support	$ 58,600	$ 48,100	$ 48,200
(c) Indirect Expenses *	31,600	21,800	19,300
TOTALS:	$ 90,200	$ 69,900	$ 67,500

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

11. PUBLICATIONS DURING FY 1972

Hays, E. F.: Morphology of Thymic Grafts Exposed to Lymphomagenic Virus, J. Surg. Oncology 3:517-523 (1971).

Hays, E. F.: The Effect of Actinomycin D on Lymphomagenesis of Thymic Grafts Exposed to Murine Leukemia Virus (MuLV), 14th Annual Meeting, American Society of Hematology, San Francisco, Calif., Dec. 5-7, 1971.

Hays, E. F.: Graft-Versus-Host Reactions and the Viral Induction of Mouse Lymphoma, Cancer Res. 32:270-275, Feb. 1972.

Hays, E. F.: Development of Neoplasia and Karyotype Analysis in Mice with Graft-Versus-Host Reaction, Cancer Res. 32:276-279, Feb. 1972.

12. SCOPE OF THE PROJECT

Factors Influencing Lymphomagenesis in the Mouse Thymus

The thymus is the target organ for the induction of lymphoma by murine leukemia virus (MuLV), Gross type. The AKR mouse has a high spontaneous incidence of thymic lymphoma and its onset can be accelerated by inoculation of young mice with MuLV. When the thymus is exposed to MuLV in vitro, and grafted to thymectomized hosts, the grafts develop lymphoma. This project is designed to study this system of viral lymphomagenesis by determining the effect of ionizing radiation, inhibitors of DNA synthesis, transcription, protein synthesis, and reverse transcriptase (RNA dependant DNA synthesis) on the development of malignancy in thymic grafts. The reverse transcriptase has been shown to be present in C-type RNA oncogenic viruses, such as MuLV, and it is believed to be a mechanism for insertion of viral genetic material into the host cell genome. Evaluation of each of the above factors will be made on thymic grafts at the time of initial viral infection, and in those with established infection of 24 hours or 3 weeks. The purpose of this work is to learn more about the mechanism of viral induced malignant transformation of cells in vivo.

Immunologic Responsiveness and Lymphomagenesis

Host responses to viral and tumor antigens play an important role in lymphomagenesis by MuLV. Since the thymus is the target organ for lymphoma development, and also the reservoir for lymphocytes that participate in cellular immunity, the immune responsiveness of MuLV infected mice prior to lymphoma development is of importance. Research has been accomplished in this project which has shown defective cellular immune responses in AKR mice as measured by two parameters. The questions are being asked as to whether such a defect is responsible for the unique susceptibility of the AKR mouse to lymphoma, and whether this animal is capable of an immune response to viral and cellular antigens. The present literature yields conflicting results on this point. Furthermore, since there is an increased incidence of lymphoma in human diseases with suppressed immune reactivity, such animal studies as outlined above have increased relevance.

There is also some evidence from patient observations that lymphoid neoplasia might result from prolonged increased immune reactivity. Animal studies producing a state of increased immune reactivity in the presence of MuLV are part of this project. They are designed to determine if altered immunologic status in the host has an effect on viral lymphomagenesis.

Lymphoma Cell Transfers

Growth characteristics of in vivo cloned mouse lymphoma cells (Vredevoe, 1970) are being analyzed. 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.

Colony Forming Cells in Mouse and Human Bone Marrow

Both human and mouse bone marrow when cultured in soft agar, form colonies of granulocytic and monocytic cells under the influence of colony stimulating factor from various sources. Studies are being carried out to look at numbers and types of colonies formed from marrow of intact and thymectomized mice exposed to MuLV before and after development of the neoplastic disease caused by this virus. These marrow cells will be fractionated on a bovine serum albumin density gradient, to more precisely define the precursor cells involved in colony formation. Studies of colony forming ability will also be carried out using intact and fractionated marrow from normal individuals and from patients. The objective of this research is to study the stem cell or cells involved in colony formation by initially looking at colony forming ability of mouse bone marrow, in the presence of leukemogenic virus and during its replacement by neoplastic cells. Its purpose is also to study colony forming cells with the same technics in the presence of leukemia and other hematologic disorders in man.

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 1972

Factors Influencing Lymphomagenesis in the Mouse Thymus

Studies using ionizing radiation to evaluate viral lymphomagenesis in thymic grafts, have shown that irradiation inhibits the initiation of viral infection, as well as the progression of an established infection in the thymus, but that a heavily irradiated thymic graft can develop lymphoma when placed in a virus infected host. The following metabolic inhibitors are under study in this thymic graft system: cytosine arabinoside, an inhibitor of DNA synthesis; actinomycin D, an inhibitor of DNA dependant RNA synthesis; AF/APB, a rifampicin derivative and streptovaricin complex, both of which inhibit reverse transcriptase; and puromycin, an inhibitor of protein synthesis. To date, studies have shown that thymic grafts exposed to all these inhibitors are able

to regenerate when placed under the kidney capsule. More complete studies are available with actinomycin D, and have shown that grafts exposed to this drug were able to restore the defective immune response of thymectomized hosts. Actinomycin D inhibited lymphoma development in thymic grafts when such grafts were exposed in vitro to this compound just prior to MuLV, as well as after viral infection had been established in vivo for 24 hours. Actinomycin D incubation did not inhibit lymphoma development in thymic grafts placed on hosts previously infected with MuLV.

It is concluded that inhibition of lymphoma in these experiments is the result of actinomycin D preventing the establishment of viral infection in thymic cells, and not to a functional alteration of the thymus produced by direct cytotoxic action of the drugs, thus implying that DNA transcription is an essential step in lymphomagenesis with MuLV.

Immunologic Responsiveness and Lymphomagenesis

The role of an increased immune reactivity produced by graft-versus-host reaction (GVH) in the development of lymphoma was studied in mice carrying MuLV and the genetic susceptibility of lymphoma development. GVH reactions were produced by inoculating parental spleen cells into CBA/H T_6T_6 x AKR and SJL/J x AKR/F_1 hybrids. The spleen cells were obtained from animals with and without previous exposure to MuLV. Thymic lymphomas were found to develop in high incidence in mice receiving spleen cells from virus inoculated parental donors. The lymphomas were of host cell types. Manipulations designed to increase the intensity of the GVH reaction were shown to have no relationship to the development of lymphoma. It was also demonstrated that AKR cells had an impaired ability to produce GVH reactions and yet were highly lymphomagenic when they came from virus inoculated donors. It was concluded that lymphomagenesis was directly related to the presence of virus in the spleen cells and not to the GVH reactions resulting from their inoculation. A second group of experiments using hybrid mice of a cross between parents with a high incidence of reticulum cell sarcoma and parents containing a marker chromosome were carried out. No evidence was found in the mouse strains tested to support the hypothesis that the immunologic disorder associated with a GVH reaction could eventuate in lymphoma.

Lymphoma Cell Transfers

Studies of the transplantation growth characteristics of low doses or single cell isolates from Gross virus-induced lymphomas have been continued. Growth characteristics of lymphoma cells from primary transplants (first transfer) and long transplanted lines were compared between and within the following mouse strains: 1) AKR, a mouse strain with a high incidence of spontaneous lymphoma, 2) C3H/HeJ, and 3) CBA/T_6 (donor) to CBA/H (recipient), the C3H/HeJ and CBA/H strains with a low incidence of spontaneous lymphoma. Findings have been 1) that in C3H/HeJ mice there is a significantly lower incidence of lymphomas in syngeneic recipients of low doses of long transplanted lymphoma cells as compared to recipients of low doses of cells from primary transplants; this difference in incidence cannot be shown in the AKR strain; 2) that there is a significantly higher LD 100 dose for primary lymphoma cells transplanted syngeneically in C3H/HeJ as compared to syngeneic transfers in AKR mice; 3) that single cells can develop into lymphomas when such cells are derived from long transplanted lymphomas in C3H/HeJ, CBA/H and AKR mice, but only from primary transplants of lymphomas in AKR mice, and that such lymphomas can be maintained by syngeneic transplantation; 4) that all single cell transplants developing into lymphomas in the CBA/H recipients carry the T_6 donor chromosome marker in the lymphoma developing in the recipient; 5) that Gross virus cannot be demonstrated on long transplanted C3H/HeJ or CBA/H

lymphomas tested by AKR newborn bioassay; virus can be demonstrated in primary transplants of lymphomas in C3H/HeJ and AKR mice.

Colony Forming Cells in Mouse and Human Bone Marrow

In collaboration with Charles G. Craddock, M.D., Professor of Medicine, UCLA School of Medicine, studies of colony forming capacity of mouse and human bone marrow cells in soft agar have been continued. In all instances, serum from C57 Bl mice removed after intravenous inoculation of endotoxin 4 hours previously, has been the source of colony stimulating factor (CSF). We have found that an addition of such serum to mouse bone marrow cells is essential to the development of colonies which begin at 3 days and are fully developed by 7 days. Colonies rarely develop without the addition of CSF. This was not found to be the case in human bone marrow where colonies develop in the absence of CSF. Mouse CSF did increase the number of colonies formed. The number of unstimulated colonies seemed to be related to the percentage of granulocytes in the marrow preparations. The number of colony forming cells was greater in mouse than in human marrow. The colonies of human marrow at 7 days were composed of monocytes and granulocytes, whereas those in mouse marrow at 3 days are mixed granulocytic and monocytic, but are pure monocytic colonies at 7 days.

15. EXPECTED RESULTS IN FY 1973

The studies of the effect of metabolic inhibitors on lymphomagenesis in thymic grafts will be continued. In every instance the evaluation will be of: 1) graft incubated in inhibitor and then exposed to MuLV in vitro, 2) graft exposed to MuLV for 24 hours in vivo, then incubated with metabolic inhibitor in vitro, 3) graft incubated with metabolic inhibitor in vitro and placed in hosts with established viral infection, 4) grafts incubated with MuLV in vitro, grafted for 3 weeks, removed, and divided. Half of the graft will be exposed to metabolic inhibitor, the other half to tissue culture medium and each reimplanted in secondary hosts. The regenerative capacity of inhibitor incubated grafts has been evaluated as indicated above; further functional capacity of the grafts will be evaluated by grafting inhibitor incubated grafts into thymectomized, irradiated adult animals and determining their ability to restore the effective and defective immune response in these animals. The tissue culture assay for MuLV, using the UV XC test has been adapted to our laboratory conditions. Quantitative data on the virus preparations that are used in this study will be obtained from this assay. The immune responsiveness of AKR mice will be further evaluated by studying their ability to develop adoptive immunity to the antigens of MuLV and tumors induced by MuLV. The responsiveness of AKR mice will be contrasted to that of CBA/T_6 mice.

Single Gross virus-induced mouse lymphoma cells or low doses of such cells will be isolated and transplanted to syngeneic recipients of the three types used previously: AKR; C3H/HeJ; CBA/H (CBA/T_6T_6 donor lymphoma).

Single cells will be isolated and transplanted in a semisolid gelatin medium (Vredevoe, 1970). Lymphoma donor cell populations will be sorted as to: a) virus content (UV-XC and newborn assays), b) morphology of cells, c) histology of lymphoma. These characteristics will be analyzed in relation to incidence and latent period of lymphomas developed from single cells derived from such a characterized population. Clones of single cells arising from a characterized population will again be analyzed by the three assays in relationship to incidence and latent period. This is done to determine the nature of the population from which single cell clones can be derived and the single cell itself that develops into frank lymphoma. The broad objective is to

determine characteristics of cells which are capable of developing lymphomas upon transfer at low doses or as single cells.

Success of immunotherapy with BCG will be compared among the syngeneic characterized mouse lymphomas. The objective will be to determine those tumor cell characteristics which are correlated with successful immunotherapy.

The colony forming ability of marrow from pre-leukemic and leukemic AKR mice will be evaluated. The technic of separating marrow cells by density gradient centrifugation will be perfected. Mr. Thomas Oberjat, Staff Research Associate II at the Laboratory of Nuclear Medicine and Radiation Biology, will carry out these studies. The effect of neonatal thymectomy in C3H mice on the colony forming capacity of bone marrow will be evaluated. Thymectomized, virus infected AKR mice, which do not develop lymphoma, but could be expected to develop myeloid leukemia, will be studied at intervals prior to leukemia development. Studies of colony forming capacity of both normal and diseased human bone marrow will be continued with special emphasis on alterations occurring in leukemia and lymphoma and their relationship to therapy of the disease states.

16. EXPECTED RESULTS FY 1974

We hope by 1974 to have results from our studies with metabolic inhibition which will give us specific information about virus cell interactions leading to lymphoma.

It may be possible to relate virus expression by lymphoma cells to growth characteristics and response to immunotherapy. The transfer of single cells is an interesting approach to the study of chemotherapeutic and immunotherapeutic agents. By such transplants it may be possible to project the stages of lymphoma development within the host and correlate this to success of chemo-or-immunotherapy.

The bone marrow studies will provide data regarding the nature of marrow stem cells and their alteration in disease states effecting the marrow.