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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

8503073

COVER PAGE
(THIS PAGE MUST BE THE FIRST PAGE OF THE APPLICATION)

1. Name of Institution: University of Washington
2. Address: City Seattle 3. State WA 4. ZIP 98195
5. Principal Investigator: Cornelius Rosse, M.D., D.Sc.
6. Department: Biological Structure
7. Telephone: Area Code 206 Office: 543-1860 Home: 329-6408
8. Title of Application: Gammacell-40 Irradiator
9. Research Area: Health and Environmental Impact of Energy-Related Chemicals
10. Total DOE Funding for Research in Selected Area, (During the last two calendar years): $ 868,085
11. Estimated Purchase Cost of Equipment: $ 129,628
12. Amount Requested from DOE: $ 103,703

List all Federal agencies which are currently considering proposals from the institution involving the same or similar equipment.

13. Agency: none Agency Proposal Number: N.A.
14. Agency: none Agency Proposal Number: N.A.

NOTE: The institution is responsible for informing DOE if a proposal involving the same or similar equipment is submitted to a federal agency prior to the announcement of DOE's URI awards.

15. List any federal agency which has provided funds to the institution during the past two years for the same or similar equipment.

Agency: none Amount of Funds: none

16. Please check one of the following :

[X] I authorize external review of this proposal.

[ ] I do not authorize external review of this proposal.

Signature of Principal Investigator: C Rosse Date: 12-4-84

Name and Title of Institutional Official (President or Designee)

Donald W. Allen, Director

Grant and Contract Services

Signature: Donald W. Allen
Date: 12/20/84

Name and Title of Financial Officer

Donald W. Allen, Director

Grant and Contract Services

Signature: Donald W. Allen
Date: 12/20/84

REPOSITORY Oak Ridge Operation Records Holding Area
COLLECTION Documents 1944-94
BOX No. B-77-5 Bldg. 2714-H
FOLDER Univ. of Washington Grant No. DE-FG05-85ER 75228

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U.S. DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM

BUDGET PAGE

ESTIMATED COSTS

Instrumentation	Requested of DOE	Institution's Share *(1)*	Other Federal Funds *(2)*	TOTAL
A. Purchase Price *(3)*	103,703	3,925	-0-	107,628
B. Estimated Cost Sharing	X X X X	22,000	-0-	22,000
(Shipping, installation, etc.)				
C. TOTAL	103,703	25,925		129,628

NOTES: *(1) Non-Federal funds only*

(2) Estimate funds to be obtained from other Federal agencies for purchasing the instrument, user charges, etc.

(3) Only the purchase price of the instrumentation is eligible for DOE funding through this program.

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A. **Purchase Price (Detail)**

	Description	Quantity	Total Estimated Unit Price	Total
1.	Gammacell-40 Self-Contained Irradiator	1	$ 61,830	$ 61,830
2.	Dual Sources - Caesium-137	1	28,505	28,505
3.	Attenuators	1	2,565	2,565
4.	Sample Cavity Collimator	1	3,875	3,875
5.	Extra Sample Drawer Tray	1	60	60
6.	U.S. Customs duty, 4.6%, items 1 - 5, excluding item 2, Dual Sources			3,143
7.	Washington State Compensating Sales Tax, .079, items 1 - 5			7,650
	Subtotal:			$107,628

B. **Estimated Cost Sharing (Detail)**

		Institution's Share	Other Federal Funds	Total
1.	**Shipping/Handling** *	$ 4,500		$ 4,500
2.	**Installation**			
3.	**Building/Laboratory Renovation** **	$ 17,500		$ 17,500
	Subtotal:	$ 22,000		$ 22,000

* Shipping Cost - 6,800 lbs., Ottawa, Canada - Seattle, WA $ 3,000
Local cartage, unloading, moving into Building 1,500

** Floor reinforcement to distribute weight, and removal and replacement of doors to admit equipment

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DEPARTMENT OF ENERGY
UNIVERSITY RESEARCH INSTRUMENTATION PROGRAM
CONTRACT SUMMARY FORM

Contract/Subcontract No.	Principal Investigator	Title	Contract Dates FM MO	Contract Dates FM YR	Contract Dates TO MO	Contract Dates TO YR	Total Award Value	$ AMT Awarded for period 19 to 19	DOE Technical Monitor/Location
DE-AT06-79EV10270, MD10	Cornelius Rosse	Marrow-Tumor Interactions, The Role of Bone Marrow in Controlling Chemically Induced Tumors	01	84	12	85	450,378		
DE-AT06-82ER60069, MD1	Lawrence A. Loeb	DNA Damage in Human Cells	09	82	08	85	277,707		
DE-AT06-81ER10879, MD4	Benjamin D. Hall	Genetic Engineering of Yeasts for Fermentation of Xylose to Ethanol	04	84	03	86	70,000		
DE-AT06-80-ER10680, MD3	Mary E. Lidstrom	Genetics in Methylotrophic Bacteria	02	84	01	87	70,000		
							$868,085		

*There are the DOE-Sponsored Grants and Contracts pertaining to Health and Environmental Impacts of Energy Related Chemicals in the School of Medicine, University of Washington. 81 additional Grants and Contracts are sponsored by DOE at the University of Washington.

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RELATED FEDERAL AGENCY CONTRACTS

Agency	(Grant No.) Contract No.	Title	Contract FROM MO	Contract FROM YR	Dates TO MO	Dates TO YR	Total Award Value
NIH	CA 20823	Cornelius Rosse, MD DSc Lymphocyte Production and Traffic in the Bone Marrow	4	80	3	85	$ 394,424
NIH	AI 19696	Lai-Ming Ching, PhD Cytotoxic T Lymphocyte Precursors In Vitro	1	83	12	85	103,700
NIH	AG 04360	Andrew Farr, PhD Age Dependent Modulation of T Cell Function	7	83	6	86	175,473
March of Dimes	5-455	Brian Hamilton, MD PhD Bone Marrow Transplantation-Effect of Graft-versus Host Disease on Immunologic Reconstitution	9	84	8	86	50,000
NIH	CA 32757	Brian Hamilton, MD PhD Mechanisms of Minor-H Antigen GVHD	7	82	6	85	107,500
NIH	CA 39889	Brian Hamilton, MD PhD Marrow Transplantation: Immune Dysfunction in GVH	1	85	12	87	285,509
NIH	CA 38189	Minako Lee, MD Bone-Bone Marrow Interaction	6	84	5	87	187,707
NIH	CA 32553	Sylvia Pollack, PhD Specific Anti-Tumor Activity of Armed Lymphoid Cells	12	83	11	87	353,448
NIH	CA 37006	Sylvia Pollack, PhD Regulatory Interactions of NK Cells with B Cells	3	84	2	87	204,254

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RELATED FEDERAL AGENCY CONTRACTS

Agency	(Grant No.) Contract No.	Title	Contract FROM MO	Contract FROM YR	Dates TO MO	Dates TO YR	Total Award Value
NIH	AG 01751	Norman S. Wolf, DVM PhD Gene Action in the Pathobiology of Aging	8	84	7	85	80,771
NIH	CA 24845	Lawrence A. Loeb, MD PhD The Fidelity of DNA Replication in Human Lymphocytes	1	84	12	85	146,459
EPA	B 623010	Lawrence A. Loeb, MD PhD DNA Damage and its Expression in Mammalian Cells	6	84	5	85	63,874
NIH	AG 01751	Lawrence A. Loeb, MD PhD Gene Action in the Pathobiology of Aging	8	84	7	85	44,756

5 (continued)

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SUMMARY

a. This application requests funding for the purchase of a Gammacell 40 Laboratory Irradiator manufactured by Atomic Energy of Canada, Ltd. The equipment is widely used in research programs in cellular and molecular biology throughout North America. The equipment is needed to enhance seven research programs located in the Departments of Biological Structure and Pathology, School of Medicine, University of Washington. Three of these programs receive direct support from DOE and four are funded by a number of NIH grants.

b. The irradiator will be used in the following types of projects:

i. Elucidation of the mechanisms through which Natural Tumor Growth Regulatory Cells (NTRC) generated in the bone marrow participate in cellular defence against cancer. The tumor neutralization potential of these cells will be assessed in the Winn assay by transferring cell mixtures into irradiated mice.

ii. Investigation of the mechanisms through which certain neoplasms influence and modulate hemopoietic stem cell and progenitor cell populations including those that contribute to anticancer host defense. Bone marrow cells of tumor-bearing mice will be assayed in irradiated recipients for pluriopotent stem cells, B and T lymphocyte precursors and NK cell precursors.

iii. The maturation of natural killer (NK) cells will be studied in the bone marrow. Cell transfer experiments into irradiated mice will assess the developmental potential of defined precursor populations from the bone marrow.

iv. The role of mature NK cells will be investigated in the regulation of the antibody response against antigens. Irradiated animals will provide radiation-resistant NK cells for *in vivo* and *in vitro* studies of the primary antibody response.

v. The effect of aging on the thymic microenvironment by assessing Ia antigen expression will be investigated. Bone marrow chimeras will be used to define the cell population responsible for maintaining or restoring Ia expression in the thymus.

vi. The genetic resistance or susceptibility to diabetogenic agents will be investigated in appropriate mouse strains. Irradiation is needed to eliminate suppressor or T cells during adoptive transfer of diabetes by spleen cells and also in

the creation of bone marrow chimeras that provide information about the identity of the cell population upon which susceptibility or resistance depends.

vii. The role of minor histocompatibility antigens will be investigated in the immunobiology of bone marrow transplantation in acquired immunodeficiency syndrome.

viii. The irradiator will be used in several bone marrow transplantation studies that investigate the depletion of the reserve in hemopoietic stem cell potential, the effect of aging on the hemopoietic microenvironment and the self-replicating ability of hemopoietic stem cells.

ix. It will be investigated to what extent various agents alter the accuracy of DNA replication. In vitro systems are used that copy DNA with the accuracy similar to that achieved in cells.

x. Experiments will determine the effect of elevated temperatures on DNA repair by assessing the rate of single-strand rejoining in human lymphocytes that have been exposed to irradiation.

c. Acquisition of the Gammacell 40 will have a very positive impact on both the quantity and quality of research performed in experimental hematology, tumor immunobiology and molecular biology. The rate of progress in all studies is limited at present by the lack of an adequate irradiator for experimental work. The Gammacell 40 will reduce the possibility for Elkind repair and will fully meet the needs of the 7 programs described in this application.

d. Graduate student participation as research assistants is an integral part of all the programs described. The number of graduates from Biological Structure is shown in Appendix II. There are 14 students currently enrolled whose work will immediately benefit from acquisition of the irradiator.

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NARRATIVE

Commitments, Accomplishments and Potential of the Program in the Area of HEALTH AND ENVIRONMENTAL IMPACTS OF ENERGY-RELATED AGENTS

Commitments. The Department of Biological Structure at the University of Washington has held a research contract with the Department of Energy and its predecessors (ERDA, AEC) uninterruptedly for over 20 years. Until 1979 the Principal Investigator of the successively renewed contracts was Dr. Newton B. Everett, Chairman of the Department of Biological Structure. The focus of the "DOE"-funded research was **lymphocyte biology and hemopoietic stem cell-related research.** The Principal Investigator of this URI application (Cornelius Rosse) became involved with this research as a co-investigator in 1968 when he was still a junior Assistant Professor. He became responsible for increasing segments of the DOE funded investigations; in 1979, following the death of Dr. Everett, he assumed the responsibility for the then current contract. In 1980 a new contract was awarded by DOE to the PI of this URI application with the new focus of elucidating **cellular defense mechanisms of the host that are engaged in the recognition and neutralization of environmentally induced neoplasms.**

In 1981 the PI was appointed Chairman of the Department of Biological Structure, and in the reorganization and rebuilding of the Department he has given high priority to strengthening the DOE-funded component of the Department's research program:

1. Three individuals were appointed to the research faculty and two to the regular faculty who were recruited because of their expertise in tumor biology, cellular immunology and hemopoiesis in order to create a multifaceted interactive program that is well equipped to study host defense against cancer on a fundamental and broad level;

2. The PI obtained a construction grant from the National Cancer Institute and matching funds from the School of Medicine, University of Washington to rebuild, expand and re-equip over 4,000 square feet of laboratory space, specifically designed to meet the needs of the DOE-supported research program. This facility, called the Biological Structure Cancer Research Labortory, accommodates all the co-investigators on the PI's current DOE contract and no other member of the Department occupies space in this

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facility. All the co-investigators on the DOE contract have, since their appointment, obtained NIH-funded grant support for components of their research. These components represent outgrowths of the DOE-supported program and remain closely integrated with it.

In addition, a DOE-sponsored research project was established in 1982 in the Department of Pathology, Principal Investigator Dr. Lawrence Loeb.

This brief account of the long history of DOE-sponsored research in the Department of Biological Structure should indicate not only the impressive growth of the program but also the substantial, long-lasting and tangible commitment of the Department and of the School of Medicine to this program.

Accomplishments. It is largely through the DOE-sponsored program that members of the Department of Biological Structure have made important contributions over the years to the understanding of lymphocyte biology. Some of the major accomplishments that have contributed to the recognition our program has received are listed below:

1. This laboratory pioneered the use of radioactive DNA precursors for the study of hemopoietic cell production and especially lymphocyte production. These techniques have led to the definition of different populations of lymphocytes in terms of their life spans and rates of turnover.

2. It was in this laboratory in the 1960's that short- and long-lived lymphocytes were first described, a concept that had broad implications for understanding the life span of lymphocytes and their functions.

3. It was in this laboratory that the bone marrow was identified as a primary source of lymphocytes, a concept that has now become central in cellular immunology.

4. Work in this laboratory has focused attention on the relationship of lymphocyte production to the production of nonlymphoid hemopoietic cells in the bone marrow.

5. We have demonstrated the bone marrow's contribution not only to the peripheral B lymphocyte pool, but have identified the precursors of T cells in the bone marrow and our current work continues to investigate the role of bone marrow pre-T cells in thymic and extrathymic T lymphocyte production.

More recent accomplishments are presented in Summary of Major Accomplishments (p. 30).

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Potential. Because of the focus of the DOE-sponsored program on such fundamental phenomena as cell differentiation, cell production, cell migration and cell interactions, DOE-sponsored research exerts a significant influence on departmental research programs concerned with the cell biology of the intercellular matrix, fetal skin biology, cell differentiation in skeletal muscle and connective tissue, mechanisms of cataract formation in the lens of the eye, reproductive biology and macromolecular structure.

The overall environment in the Cancer Research Laboratory, as well as in the whole Department, is very conducive to innovation and to the adaptation and exploitation of new techniques and approaches. Many members of the group and of the whole faculty are only just beginning to realize their potential in research and academia; this is being borne out by the continuing success in obtaining funding for newly submitted grant applications.

Description of Equipment Requested and the Need for It

Description. This application requests funding for the purchase of a low dose rate laboratory irradiator to be used primarily in research programs of cellular and molecular biology, which are located in the Biological Structure Cancer Research Laboratory, Department of Biological Structure and the Department of Pathology, School of Medicine, University of Washington. The instrument of choice is the Gammacell 40 low dose rate laboratory irradiator manufactured by Atomic Energy of Canada, Ltd., Ottawa, Canada. The detailed description and specifications of this unit are attached (Appendix I). The Gammacell 40 is a self-contained irradiator widely used in biological and physical science laboratories throughout North America (see Appendix I).

The research supported by the DOE contract relies heavily on irradiated rodents for studying the lineages, regenerative potential, migration pathways and various interactions of cells that participate in the defense of the host against cancer. The availability of an efficient, reliable irradiator that provides a uniform field of irradiation is essential for meeting the objectives of our research. The lack of such an instrument and our limited access to the available next-best choice are currently

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presenting a serious obstacle to our work. We have tried to obtain the Gammacell 40 by a supplemental application to our contract for the past two years, but curtailments in the DOE budget have prevented funding of this application despite the fact that our need has been recognized by the central office (for reference contact Dr. George Stapleton, Health Effects Research Division, ER-72 GTN, Office of Energy Research, Tel: (301)-353-5039). **The URI program is precisely the type of mechanism that is sorely and urgently needed to provide for our need. Moreover, acquisition of the irradiator through the URI program will substantially benefit and enhance a number of research programs in Biological Structure and Pathology that are not directly sponsored by DOE.**

Need. The following are the specific reasons for the need to purchase a laboratory irradiator for our research programs:

1) The only available irradiator for biological research at the University of Washington Health Sciences is a Picker therapy clinical Cesiumm-137 (Cs-137) irradiator that has been converted by the Department of Radiological Sciences, School of Public Health and is maintained by that department.

2) The output of this machine at the distance required to irradiate groups of mice is 45-50 R/minute. At this low delivery rate, Elkind repair is known to occur in hemopoietic stem cells, which introduces an element of uncertainty into comparing our experimental data with those of other laboratories. These physical limitations also impose less than optimal conditions for preparing feeder layers in cell cloning experiments and for DNA replication studies.

3) The machine operates with a single Cs-137 source, irradiating the mice from the dorsal aspect, which results in a different biologic effect than dorsal-ventral irradiation from a dual source.

4) A non-uniform field is delivered that varies 10% over the length of a mouse.

5) The machine lacks self-shielding and its operation is complex and time consuming.

6) The source-subject distance must be set each time the unit is used, requiring recalibration; this leads to interexperimental variation in dose rate/dose.

7) Being the only available machine for experimental use, it is so heavily booked

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that scheduling must be done weeks in advance, and there are many conflicts.

8) User fees, in excess of $2000-3000/year for each of the groups are required to maintain our research at the projected level.

Benefits. The Gammacell 40 offers the following advantages for our research programs:

1) The irradiator has been specifically designed for the type of biological research performed in our programs.

2) The dose rate is 140 R/minute, shown to be the optimal for producing biological effects required in our research.

3) Two opposing Cs-137 sources provide improved uniformity in the exposure field.

4) The instrument is fully self-shielded, obviating remodeling and shielding of the room where it will be in use.

5) A built-in ventilation system delivers air to the mice during irradiation.

6) Maintenance costs are low; 30-year isotope half-life reduces replacement costs.

7) Operation and maintenance do not require specially trained personnel, and a high degree of safety is assured for the operator.

8) Several of us have used the Gammacell 40 at other institutions and are familiar with its advantages for the type of work we perform.

Alternative Equipment. Other instruments we have considered include: Cobalt 60 units (Gammacell 200 or Gammacell 500) and electric x-ray machines from Westinghouse or Philips. The disadvantages of the cobalt units are the high dose rates delivered and the relatively short half-life (5.7 years). The disadvantages of using electronic x-ray sources have been discussed recently by Uphoff (38). She found significant differences in results of bone marrow transplantation experiments in mice when she compared Westinghouse and Philips x-ray units, even when the dose rates were held constant. The qualitative differences in x-ray effects were ascribed to changes in the high voltage lines, a problem obviated by using isotope sources. Uphoff also discussed the advantages of using dose rates in the neighborhood of 140 R/minute and the need for opposing sources rather than a single source for delivering uniform biological effects.

Of other commercially available units, we have extensively evaluated the Mark-I-

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Cesium-137 irradiator manufactured by J. L. Shephard Co., Glendale, CA. The design of this machine has several significant disadvantages compared to Gammacell 40. The most important of these is the mobile source. A recent extensively published accident with this instrument that resulted from failure of lowering the source when the radiation chamber was opened by the operator is a regretable but noteworthy illustration of our concerns and provides ample justification for selecting the Gammacell 40.

In summary, there is a definite need for a laboratory irradiator for attaining the objectives of the research programs described in this application. **Gammacell 40 meets the needs of all these research programs for the foreseeable future, and it is the most suitable instrument currently available for this purpose.** Acquisition of a Gammacell 40 will not only assure that the research programs can proceed at an improved pace, but will provide optimal conditions for irradiation of animals and cells upon which the interpretation of much of our data depends.

Description of Research Sponsored by DOE and Other Federal Agencies

The research programs of the Biological Structure Cancer Research Laboratory are concerned with the differentiation of various cell types in the hemopoietic and immune systems. Five specific programs can be defined that include several subprojects and that interdigitate extensively with respect to their objectives, experimental approaches and significance. The sixth program (P.I., Wolf) in Pathology is closely allied with some aspects of research in the Biological Structure Cancer Research Laboratory and there is substantial collaboration between the programs as evidenced by publications. The seventh program (P.I., Loeb) in Pathology is not directly related to the programs in Biological Structure. The grants that support this research activity are shown in "Related Federal Agency Contracts" (p. 5).

It will be evident from the brief descriptions of the programs that all of them rely heavily on the use of an irradiator, and that the current circumstances force us to perform our research under less than optimal conditions. The Gammacell 40 requested in this application is specifically designed for the type of research we are engaged in and its acquisition will have tangible and positive impact on all programs described below.

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Research Program No. 1: Project A: Marrow-Dependent Tumor Neutralization

Project B: Lymphocyte Production and Traffic in the Bone Marrow

Principal Investigator: Cornelius Rosse, M.D., D.Sc.

Co-Investigators: Lai-Ming Ching, Ph.D.
Brian Hamilton, M.D., Ph.D.
Minako Y. Lee, M.D.
Andrew Farr, Ph.D.

The major goal of research in this program is to investigate the involvement of cells derived from the bone marrow in various cellular immune responses, including the neutralization of immunogenic neoplasms. This research is currently supported by a contract awarded by the Department of Energy (P.I., Rosse); two research grants from the National Cancer Institute (P.I., Rosse and Ching), with decision on an additional NIH grant pending (P.I., Ching).

Project A

Our work during the past few years (35-37) has shown that there is a radiation-sensitive cell population present in the bone marrow of normal, unsensitized mice upon which the effectiveness of tumor antigen-specific cells largely depends in neutralizing methylcholanthrene (MCA)-induced sarcomas. We have named these cells Natural Tumor Growth Regulatory Cells (NTRC) because they exist in normal unsensitized mice and their elimination seriously incapacitates the tumor neutralization potential of tumor antigen-specific immune cells.

The specific research aims of this project address two fundamental questions:

1) The differentiation of progenitor cells, derived from the bone marrow, into immunocompetent cells under the influence of tumor antigen-T cell interaction.

2) The definition of cell interaction mechanisms that are responsible for successful neutralization of immunogenic neoplasms *in vivo*.

These aims are pursued in an *in vivo* experimental system using the Winn assay (41) in mice. This assay is the most widely used *in vivo* system for investigating questions in tumor immunobiology and is particularly well suited to our research aims.

For routine experiments, mice receive 900 R whole body irradiation and are

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transplanted subcutaneously with tumor cells. The rate of tumor growth is monitored by measuring the size of the tumor at appropriate intervals. The influence of various cell types or cell mixtures on the rate of tumor growth is assessed by admixing these cells with the tumor cells at the time of transplantation or by injecting the cells independently of the tumor cells, intravenously. The experimental strategy calls for several modifications of this basic procecure; all experiments, however, must include irradiated control animals.

Since each experiment consists of several groups of mice, with 10 mice in each group, most experiments require 50-100 irradiated animals. At present, the major limiting factors in proceeding at the appropriate pace with these studies are 1) scheduling of the available irradiator, 2) the cost of using the irradiation facility, and 3) lack of uniform exposure with the available irradiator. Since we cannot rely on a uniform field of exposure with the available Cs-137 source, interpretation of the experimental data may be questionable because of the survival of some cell types we had assumed to have been eliminated by the total body exposure.

Project B

Interest in this laboratory has centered on the production and differentiation of bone marrow lymphocytes (see reviews 32-34). We have contributed to the understanding of B lymphocyte development in the marrow (16,30), and much of our effort has been directed toward defining the role of T lymphocyte precursors in the marrow (2,12,28-31).

The research aims of the project address questions of: 1) thymic dependence of precursor T cells present in the bone marrow, 2) migration of these cells from the marrow to the thymus, spleen, and lymph nodes, 3) the relationship of pre-T cells of the marrow to the pluripotent stem cell (CFU-S) and other lymphocyte precursors (pre-B cells, pre-NK cells), 4) the microenvironmental cellular interactions and humoral factors that promote and restrict the differentiation of bone marrow T cell precursors.

The experimental systems used in this project include parabiotic pairs of mice, transplantation procedures, and an *in vitro* system developed to grow colonies of T cells from undifferentiated T cell precursors. The parabiotic model primarily addresses

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problems of cell migration and is combined with irradiation or thymectomy of one of the members of a parabiotic pair. Transplantation of various bone marrow cell fractions into irradiated recipients of appropriate haplotype is used to investigate stem cell-precursor cell relationship and the restriction that the H-2 haplotype of cellular inductive environments impose on developing T cells. The in vitro T cell colony system, used primarily by Dr. Ching, is ideally suited for separating the factors that regulate T cell differentiation through the interaction of phenotypically distinct cell types in the colonies (3).

Total body irradiation, or irradiation with shielding of the limbs or one member of a parabiotic pair, is an important requirement in this project. In transplantation studies, assaying bone marrow cell fractions for progenitor cell function requires large numbers of irradiated animals. Uniform exposure of the recipient mice in these studies is particularly important. In testing, the radiation sensitivity of the cell population being assayed for a particular progenitor cell function requires accurate monitoring of the dose rate. The currently available radiation facility does not meet the requirements for these experiments. The use of the facility for multiple, distinct purposes requires time-consuming adjustments, often leaving us in doubt whether we are, in fact, repeating our experiments under standard irradiation conditions.

The acquisition of the Gammacell irradiator will not only alleviate all these problems, but will greatly facilitate our work because of its unrestricted availability. Its chief advantage will be a uniform irradiation field that will permit us to interpret our data with greater confidence.

Research Program No. 2: Tumor Influence on Hemopoiesis

Principal Investigator: Minako Y. Lee, M.D.

Co-Investigators: Cornelius Rosse, M.D., D.Sc.
Sylvia B. Pollack, Ph.D.
Andrew Farr, Ph.D.

The overall goal of this research program is to investigate the influence of certain neoplasms on hemopoiesis, which perturb the differentiation and rate of production of various hemopoietic cells and cells potentially capable of participating in cellular

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immune responses, including those directed against tumors. This research is supported by a contract from the Department of Energy (Marrow-Tumor Interactions, P.I., Rosse) and a grant from the National Cancer Institute (P.I., Lee).

We have been studying the hemopoietic effects of the transplantable CE mammary carcinoma 1460 and have shown that in mice the carcinoma causes 1) myeloid hyperplasia of the bone marrow with neutrophil production rates seven times that of normal (18,21), 2) replacement of fatty marrow in the peripheral skeleton with hemopoietic marrow (19), 3) marked fall in the production of lymphocytes in primary lymphoid organs (20), 4) increased osteoclastic activity and marked hypercalcemia (17).

The mode of action of this tumor is through the production of various factors that are being investigated. The tumor is of considerable interest because it may give insight into the mechanisms that regulate the volume of hemopoietic tissue, the cellular microenvironmental changes necessary for supporting active hemopoiesis, and the interrelation of various types of stem cells and progenitor cells in the marrow. The relevance of this tumor model to tumor immunobiology is that it may permit investigation of the mechanisms by which tumors shut off the production of certain cell types necessary for anticancer cellular responses in the host (7,35-37). One of the aims of this research is to systematically examine the influence of CE mammary carcinoma on various hemopoietic stem cells for which assay systems have been developed. The second phase of the study will investigate other tumors for their hemopoietic effects, including those on stem cells.

In the first phase of the study, bone marrow cells obtained from mice that have been bearing CE carcinoma for various periods are assayed for hemopoietic stem cells. The bone marrow cells of tumor-bearing donors are injected into normal, lethally irradiated animals, and the number of CFU-S, as well as the recovery of CFU-C, BFU-E, CFU-E and lymphoid stem cells (CFU-B [24], CFU-T [3] and NK precursors [25]) are assayed during the phase of regeneration. In another study, bone marrow cells from normal mice are transferred into irradiated, tumor-bearing mice, and the same battery of stem cell assays performed. In a modification of this latter experimental system, fractions of

bone marrow cells enriched for specific types of progenitor cells are transferred into irradiated, tumor-bearing hosts to assess the differential effects of the tumor on various stem cells. Bone marrow cell fractions enriched for various progenitor cells are obtained by making use of physical differences between various progenitor cells and also by using monoclonal antibodies, which have been developed for the identification of these cells.

These studies rely heavily on the extensive use of an animal irradiator. A gamma cell irradiator, under our own control, would significantly boost this research program both in terms of the amount of work we would be able to perform and the reliability of the data we will obtain.

Research Program No. 3 Project A: Antitumor Activity of Armed Lymphoid Cells

Project B: Regulatory Interactions of NK Cells

Principal Investigator: Sylvia B. Pollack, Ph.D.

Co-Investigators: Cornelius Rosse, M.D., D.Sc.
Claire Robles, Ph.D.
Brian L. Hamilton, M.D., Ph.D.
Andrew Farr, Ph.D.

The major goal of the research in this program is to understand the maturation and differentiation of natural killer (NK) cells and to elucidate their roles in both *in vivo* antitumor responses of the host and the regulation of immune and hemopoietic cellular responses. The component of this research concerned with *in vivo* antitumor responses is funded by the DOE contract (P.I., Rosse) and an NIH grant (P.I., Pollack) and its other components are funded by the two NIH grants (P.I., Pollack).

Project A

Although it is known that NK cells are both bone marrow dependent and bone marrow derived (7), there is essentially no information about their maturation and differentiation program. It is not known, for instance, what stage of maturity these cells reach before they are discharged from the marrow, how their precursors relate to other hemopoietic progenitor cells and how their production in the marrow is regulated. These problems are being attacked by two parallel experimental strategies:

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1) Based on preliminary data we hypothesize that NK cells in the bone marrow acquire the ability to bind to appropriate targets (YAC-1) before they develop the capacity to lyse the targets as well (26). We are using well established techniques of cellular kinetics to label DNA synthesizing NK precursors with ^{3}H-TdR in vitro and determining on radioautographs the proliferative status and turnover of cells that bind or bind and lyse NK-sensitive targets.

2) We have developed antisera that recognize antigens expressed by NK cells (27). These antigens can be used to deplete NK cells in vivo and in vitro and they also lend themselves for surface labeling of NK cells, permitting positive enrichment of these cells by the fluorescence activated cell sorter. The use of these sera is being combined with cell kinetic studies to complement the experiments described above. Both experimental strategies rely on enriching NK cells and their precursors by the cell sorter according to cell size, granularity and the binding of anti-NK or other antisera. The cell populations that are enriched or depleted for NK cells are investigated in both in vivo and in vitro antitumor responses. We are currently testing the relationship of NK cells to NTRC and in another system we are studying the mechanisms through which NK cells may influence the rate of metastasis by various tumors.

Project B

Natural killer (NK) cells can recognize and lyse not only sensitive tumor cells but also certain normal cell types such as bone marrow cells and thymocytes. Recently we and others have demonstrated that NK cells can regulate the response of B cells to antigenic stimulation.

Our kinetic studies suggest that the regulatory effects of NK cells are important during the initiation of the antibody response. The mechanisms are being explored further in in vitro culture systems under conditions of NK enrichment or depletion. On the basis of these results we will begin to dissect the complex biological role of NK cells in antitumor responses in vivo using a Maloney virus-induced sarcoma with well characterized B and T cell responses. Experiments similar to those with defined antigens will determine whether depletion of NK cells in vivo affects the magnitude

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and/or kinetics of the antibody response to primary MSV-induced sarcoma.

The studies in Research Program No. 3 rely on the use of irradiated mice in many experiments. This is especially the case in the NTRC-related studies (described in Program 1), and also in experiments attempting to investigate the reconstitution of the NK cell population in NK-depleted, irradiated hosts by various putative NK precursors isolated from the bone marrow. Since mature NK cells are quite radiation resistant, it is particularly important to perform the experiments with radiation-sensitive NK precursors under strictly controlled and reliable conditions. Moreover, irradiated mice are used as donors of NK cells in several of the experimental protocols of Project B. The Gammacell 40 will assure all these requirements.

Research Program No. 4 Project A Age-dependent modulation of T Cell Function

Project B Autoimmune Pathogenesis of Diabetes

Principal Investigator: Andrew Farr, Ph.D.

Co-Investigators: Lai-Ming Ching, Ph.D.
Cornelius Rosse, M.D., D.Sc.

The major goal of this research program is to understand how the age-related decline in T cell function depends on the modulation of the intrathymic cellular environment and how the sensitization of T lymphocytes to antigens expressed by damaged pancreatic islet cells predisposes to the pathogenesis of insulin-dependent diabetes. This program is funded by the National Institute of Aging and the Diabetes Research Center, University of Washington (P.I., Farr). Decision on an additional NIH grant is pending (PI, Farr).

Project A

There is evidence that the expression of Ia antigens on cells comprising the thymic microenvironment influences T cell differentiation and maturation. We have discovered that Ia expression in the thymus declines with age and is lost also during *in vitro* culture of the nonlymphoid elements of the thymus (5). There are two cell populations that express Ia in the thymus: epithelial cells and bone marrow-derived mononuclear cells. In order to determine the role of bone marrow-derived cells in maintaining Ia

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expression in the thymus, the intrathymic rate of turnover and life span of these cells must be determined and correlated with the antigen expression specificity of the T cells generated in the thymus. Experimentally, this is best achieved by creating bone marrow transplantation chimeras between mice of distinct H-2 haplotype in order to distinguish between thymic epithelial cells of the host and marrow derived cells generated by transplanted stem cells. In such studies, lethally irradiated B6 mice are reconstituted with B6XA (F_1) bone marrow and the cells are identified in the chimera by double labeling with appropriate anti-H^2 antisera and anti-Ia antisera. Cell kinetic studies using ^{3}H-TdR in the chimera provide the information needed about cell turnover and life span of the bone marrow-derived cells.

Project B

We have been investigating the autoimmune pathogenesis of insulin-dependent diabetes in mice treated with the diabetogenic agent streptozotocin. There are two interesting features of this experimental model:

1) The disease can be adoptively transferred by spleen cells of the diabetic animal,

2) There is a genetic basis for the pathogenesis of the disease as certain mouse strains are susceptible to streptozotocin, whereas others are not.

The study of both aspects of the pathogenesis rely on the use of an irradiator. Recipient mice of diabetic spleen cells need to be sublethally irradiated to eliminate radio-sensitive suppressor T cell populations. In other experiments lethally irradiated mice need to be used as recipients of cell mixtures of different types. Similarly, creation of bone marrow transplantation chimeras is necessary for dissociating the lymphoid and nonlymphoid elements in the pathogenesis of the diabetes. For instance, lethally irradiated streptozotocin-resistant mice (BALB/c) need to be reconstituted with bone marrow of the susceptible strain (B10.D2) and vice versa (with appropriate controls of syngeneic transfer) and the degree of hyperglycemia determined after streptozotocin injury.

The availability of a dependable irradiator is essential for both projects in this research program. One of the limiting factors in proceeding with these experiments on

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the desired scale is the limited access to the irradiator available for experimental purposes. The Gammacell 40 irradiator under our own control would significantly boost this research program and would permit us to interpret our data with greater confidence.

Research Program No. 5 Project A: Mechanisms of Graft-Vs-Host Reactions to Minor Histocompatibility Antigens

Project B: Mechanisms of Transplantation Tolerance to Minor-H Antigens

Principal Investigator: Brian L. Hamilton, M.D., Ph.D.

Co-Investigators: Cornelius Rosse, M.D., D.Sc.
Sylvia B. Pollack, Ph.D.
Hans D. Ochs, M.D.

The overall goal of this research program is to define the role of minor histocompatibility antigens (Minor-HA) in the immunobiology of murine bone marrow transplantation. Two research projects are in progress to 1) define the immunologic mechanisms that result in graft-vs-host disease (GVHD) due to Minor-HA and 2) to define the mechanism of an acquired immunodeficiency syndrome (IDS) associated with GVHD. Both projects would benefit greatly from the availability of the Gammacell 40 Irradiator requested in this application. These projects are currently funded by two grants from the National Cancer Institute (P.I., Hamilton) and an additional grant from March of Dimes (P.I., Hamilton).

The role of major histocompatibility complex (MHC) antigens such as H-2 in GVHD has been well studied as reviewed by Grebe and Streilein (6). Non-MHC Minor-HA are the primary antigens responsible for graft rejection when donor and recipient mice are matched for MHC antigens, but their role in GVHD is not well understood (13).

The P.I. of research program number 5 has developed and characterized a mouse model of bone marrow transplantation across minor histocompatibility barriers in mice which were selected for identity at the MHC (9-11). This model is particularly well suited to study the immunobiology of GVHD, including the mechanisms which result in GVHD and the mechanisms of the IDS associated with GVHD.

Project A

Initial studies have shown that GVHD in this model system is initiated by donor T

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cells (9,10). Studies are currently in progress to define the specific subpopulations of T cells which initiate GVHD and the cell populations which effect GVHD using adoptive transfer experiments. In these experiments, lethally irradiated primary recipients are transplanted with bone marrow plus spleen cells from H-2 identical donors on day 0. On day 14, when an anti-recipient response of the donor T cells can be detected in the chimeric spleen cells (8). To test the identity of the T cells capable of transferring GVHD, spleen cells from the primary recipients are selectively depleted of specific T cell subpopulations and these spleen cells are then transferred to lethally irradiated secondary recipients which are syngeneic to the primary recipients.

Project B

Graft-versus-host disease (GVHD) is frequently associated with a profound immunodeficiency syndrome (GVH-IDS). We have shown that mice with GVHD are unable to generate a normal antibody response to the T-dependent neoantigen bacteriophage Phi X 174 (39). Since the immunocompetent cells in the recipient mice are entirely of the donor type, these donor cells must acquire the immunodeficiency as a result of the GVHD. Two hypotheses have been proposed to explain the GVH-IDS: 1) The immunodeficiency results from the absence of a specific cell population, such as T helper cells of B cells, and 2) the immunodeficiency results from the presence of an excess of T suppressor cells.

The primary approach to determining which of these two hypotheses is correct is to perform a series of adoptive transfer experiments. Bone marrow and spleen cells from the primary recipients will be adoptively transferred to lethally irradiated secondary recipients which are syngeneic to the original donor strain. In this way, the chimeric spleen cells will be returned to a neutral environment in which the antigenic stimulus (minor HA) for GVHD will not exist. The immune function of the chimeric cells can then be assayed in the absence of an ongoing GVHD reaction. The first series of experiments will be done to confirm that the immunodeficiency can be adoptively transferred to the secondary recipients. In subsequent experiments, the chimeric spleen cells will be mixed with equal numbers of normal donor type spleen cells and the mixture injected into

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lethally irradiated donor type recipients. These secondary recipients will then be immunized with bacteriophage Phe X 174. If an antibody response develops in the secondary recipients, then we would conclude that the spleen cells form mice undergoing GVHD lack a specific cell population. If no antibody response develops in the secondary recipients, then we would conclude that the immunodeficiency associated with GVHD results from the presence of suppressor cells.

The experimental protocols for Research Program 5 make extensive use of lethally irradiated mice for primary and secondary transplantation experiments. The transplanted mice are then followed for survival data or used as spleen cell donors for adoptive transfer experiments or for in vitro assays of immune reactivity to Minor-HA. In addition, congenic strains, which differ from each other at single Minor-HA loci, are used as donors and recipients in transplantation experiments to define the role of individual antigens in transplantation immunity.

The studies described for Research Project 5 were initiated while Dr. Hamilton was a Postdoctoral Fellow at the Children's Hospital Medical Center and the Sidney Farber Cancer Institute in Boston, Massachusetts. Development of the basic transplantation model system, including establishment of the irradiation dose response curves for the specific mouse strains he used, took 18 months. For this work he used a Gammacell 40 irradiator located in the Joint Center for Radiation Therapy at the Harvard University Shields Warren Radiation Laboratory. Dr. Hamilton has now moved to the Department of Biological Structure in Seattle where the only animal irradiator available is a single source Cs-137 unit that has been retired from clinical service. The dose rate initially used in Boston was 122 R/minute, whereas the current source delivers only 48 R/minute with a relatively large variation over the length of the mouse (50-45 R/min from nose to tail). Consequently, the outcome of the transplantation experiments performed in Seattle has changed somewhat in that the incidence of lethal GVH has decreased, and the median survival time of mice with GVH has increased: a less severe form of GVH is currently observed. Although this observation may be due to changes in the microbial flora of the new animal facility or to differences in animal care

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techniques (bedding, food, water, etc.), the radiation source dose rate is the single greatest obvious difference. Additional experiments using this transplantation model demonstrated that the dose of irradiation is crucial to the successful engraftment of murine marrow graft recipients (23). Since the dose rate of the current source is so low, it takes considerably longer to achieve lethal doses in the range of 900-1200 R. As a result, the number of experiments actually performed is less than the number that could be done. Acquisition of a Gammacell 40 irradiator would make the current work on this mouse model more comparable with the original studies and with studies that use the model at other intensities.

Research Program No. 6: Depletion of Reserve in the Hemopoietic System

Project A: Demonstration of the Limited Self Replicability of PHSC and the Mechanisms of Limitation

Project B: The Effect of Age Differences Upon Splenic Stromal Cell Repopulation

Principal Investigator: Norman S. Wolf, D.V.M., Ph.D.

The objective of this program is to identify the pluripotent hemopoietic stem cell (PHSC) and to investigate the control mechanisms that govern its self-replication, commitment, and differentiation. This research is currently supported by a portion of a program project grant, AG-01751 from the National Institute on Aging, NIH (P.I., Martin). Two related grants have been applied for and are pending review by the NIH.

Our more recent studies have focused on establishing the limits of self-replication for the transplantable PHSC (TPHSC) and the relationship of TPHSC mitotic cycling to its differentiation (22,45). We are also studying the limits of self-replication by the stromal cells that make up the hemopoietic microenvironment, in which the TPHSC resides (42-44,46). About 95% of these experiments require the serial transplantation of mouse bone marrow cells (BMC) or other hemopoietic tissues into recipient mice that have been prepared by whole body supralethal irradiaton.

Project A

We have demonstrated that 5-6 serial transplantations are the maximum that can be carried out in (A x C57BL/6)F_1 mice with an initial finite population of isogeneic donor

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BMC (45). We are currently testing whether forcing the TPHSC to cycle before transplantation through the use of various agents reduces their capability for future transplantation.

With these and other experiments in this series, we are testing two hypotheses: 1) that there are subpopulations of PHSC with varying degrees of differentiation and self-replicative potential, and 2) that mitotic cycling, or events intimately related to cycling, produce differentiation and exhaustion of the TPHSC.

Project B

We have determined that mouse spleens that have been transplanted or had their blood vessels ligated *in situ* undergo a massive infarction followed by a rapid cellular regeneration of all internal stromal structures (42,43). This stromal regeneration is indigenous to the spleens and derives from their peripheral layer of stromal cells, which survive the infarctive anoxia. The quality of the regenerating tissue, however, is age dependent; in young adult mice there is a nearly perfect regeneration of stromal architecture, which supports TPHSC differentiation, but in old mice, scar-like connective tissue forms, and there is a loss of hemopoietic function (44,46). In future experimentation we will test whether older mice have 1) less surviving stromal cells following the infarctive event, 2) the regeneration of these stromal cells takes place more slowly, or 3) key stromal progenitor cells are insufficient in number or fail after a few cell divisions. These experiments, like those described previously, all require whole body irradiation of relatively large numbers of mice (30-90 per experiment).

Our experiments require that we expose each group of mice 22-26 minutes at a delivery rate of 46-50 R/minute. The low exposure rate is necessary with this Cs-137 irradiator because only by using a long source-to-target distance is it possible to achieve a relatively uniform dose rate over the exposure field. This exposure time allows for a certain, but unquantified, amount of Elkind recovery of the PHSC during the irradiation period, contributing to the survival of a few recipient origin PHSC that re-establish themselves in some transplant recipients. It also takes a considerable amount of time to irradite 80 or 90 mice in groups of 12 each at this exposure rate. The Gammacell 40

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irradiator requested in this grant application would remedy these difficulties, as well as solve the other problems mentioned above.

Research Program No. 7: Fidelity of DNA Replication

Project A: Mutagenic Consequences of DNA Damage

Project B: DNA Replication in X-irradiated Human Lymphocytes

Principal Investigator: Lawrence A. Loeb, M.D., Ph.D.

Co-investigators: Philip Lin, Ph.D.
Bradford Preston, Ph.D.

The emphasis of research in this laboratory is on mechanisms that guarantee the faithful copying of DNA during cell division, and deficits in these mechanisms that may lead to mutagenesis and malignancy. The studies are supported by a contract from the Department of Energy (P.I. Loeb) and several grants from NIH.

Project A

In order to understand the fidelity of DNA replication, we are reconstructing *in vitro* systems that copy DNA with an accuracy similar to that achieved in cells. In these studies, we have used mutant single-stranded ΦX174 DNA as a template, and measured the frequency with which the copied DNA is reverted to wild type DNA (40). The fact that reversion to wild type represents single-base substitutions has been verified by determining the nucleotide sequence of the product of the reaction (14). The error rates of eucaryotic DNA polymerases in copying ΦX174 DNA templates vary from 1 in 3,000 to 1 in 30,000 (15). With procaryotic systems, the accuracy achieved *in vitro* is similar to that *in vivo*, as low as 1 in 10^7. Using this system, we have ex-amined factors that alter the accuracy of DNA synthesis. With respect to this grant, we are interested in studying the effect of x-irradiation on DNA templates and on DNA polymerases, themselves. Since fidelity measurements are carried out *in vitro*, all components being defined, it should be possible to determine what chemical alterations induced by x-rays are potentially mutagenic.

Project B

In our studies with purified DNA polymerases, we have observed that DNA

polymerase-β, the enzyme presumed to be involved in DNA repair, is thermosensitive (4). We now desire to determine if this thermosensitivity results in diminished DNA repair. If so, this thermosensitivity might be relevant to the hypersensitivity of malignant cells to elevated temperature (1). We have developed assays for measuring the rate of single-strand rejoining after exposing human lymphocytes to x-irradiation (1). We will determine the effect of elevated temperature on the rates of single-strand rejoining using human lymphocytes.

The above projects require the use of x-irradiation to DNA and cells in culture. The existing facilities for irradiating cells have been inadequate. This inadequacy is most pointedly demonstrated by the difficulties encountered in scheduling the complicated protocol-type studies carried out with human lymphocytes in culture. Also, the Bio-dose rate of the current instrument requires prolonged exposure, allowing repair of DNA damage prior to analysis. Even though our proposed use of the Gammacell 40 irradiator is limited, it is the key for particular experiments.

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Summary of Major Accomplishments

Some accomplishments of DOE-sponsored research in the Department of Biological Structure have already been described in the first section of this narrative. Whereas earlier studies sponsored by DOE have defined the rates of lymphocyte production, lymphocyte life span and pathways of lymphocyte migration and circulation, more recent work has concentrated on how lymphocytes and other bone marrow-derived cells participate in cellular defense mechanisms against cancer. Although many energy-related chemicals and other agents are known to be carcinogenic and many have, in addition, deleterious effects on the hemopoietic and immune system, our programs have not concerned themselves with the investigation of any of such agents in particular. Rather, our objective has been to elucidate cellular mechanisms of the host that can participate in the recognition and elimination of cells that have been transformed by chemical agents or by neoplasia. In this approach we seek to understand the control mechanisms that govern cell production, differentiation and collaboration in a complex system.

Our notable achievements are:

1. We have discovered a cell population in the bone marrow which greatly enhances the tumor neutralization potential of tumor antigen-specific T lymphocytes. These bone marrow-derived cells are likely to play a role in such phenomena as the spontaneous regression of tumors and the elimination of tumor cells that seed through the blood stream or lymphatics, preventing the cancer cells from exhibiting themselves as metastases.

2. Our group was among the first to show that natural killer cells have an important role to play in the regulation of tumor growth *in vivo* by demonstrating that natural killer cells participate in the elimination of melanoma metastases *in vivo*.

3. We have developed antisera that permit the identification of NK cells and the assay their function both *in vivo* and *in vitro*. These reagents have been made available to investigators in this country and abroad.

4. We have demonstrated that certain tumors can exert a profound influence on the pattern of hemopoiesis, shut off the production of lymphocytes in the bone marrow and

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the thymus and greatly perturb bone metabolism. The experimental system we have developed is the best model for investigating the modulatory influences of tumors on hemopoiesis and immune responses.

5. We have developed in vitro systems that permit the study of T lymphocyte differentiation modulated by Ia expression on thymic epithelial cells and also by bone marrow-derived cells in colonies grown in semisolid media.

6. We have established an experimental bone marrow transplantation model in mice that is ideal for studying the cellular mechanisms of graft-versus-host disease and related immunodeficiency syndromes because the model duplicates the manifestations of the human disease. The model is based on minor histocompatibility antigenic differences.

7. Our studies have contributed to the understanding of the factors that may influence the self-replicating ability of pluripotent hemopoietic stem cells and the hemopoietic microenvironment that permits stem cell proliferation and differentiation.

8. We have developed in vitro systems for studying the fidelity of DNA replication and its perturbation by various agents.

Qualifications of the Faculty

The one page resumés for members of the scientific staff mentioned in this application are presented in Attachment 1. Four of these investigators are at the Assistant Professorial rank, two at the Associate Professorial rank and two are Professors. Each of these investigators is a PI of at least one peer-reviewed NIH grant and several hold more than federally supported research grants or contracts.

All members of the scientific staff mentioned in this application have conducted experiments using the currently available CS-137 irradiator, and some of them have used the Gammacell-40 at other institutions. Work described in this application does not introduce new technology or methodology into the programs of the investigators.

Institutional Cost Sharing

There are two elements with respect to institutional cost sharing:

1. The School of Medicine, University of Washington has provided the matching funds for the NCI construction grant that rebuilt the laboratory which accommodates the

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DOE-supported research program in Biological Structure. We moved into our new laboratory complex in February, 1984.

2. The University of Washington will commit funds to provide 20% of the cost of the equipment. Most of the funds provided by the University are required to meet construction costs for accommodating the irradiator. The irradiator weighs several tons, requiring the construction of floor re-inforcement and weight distribution devices in the room that is to accommodate the irradiator on the 2nd floor. In addition, installation of the irradiator will require the removal of two doors with segments of the surrounding wall so that the equipment can be moved into its permanent housing. The walls and the doors will have to be built back into their original position.

Institutional Experience with Major Research Equipment

Our electron microscopes provide the best example of major research equipment that is used by several programs in the Department of Biological Structure. A newly constructed electron microscope suite houses the following electron microscopes which serve departmental and some affiliated programs: Philips 201 TEM, Philips 300 TEM, ETEC SEM, and Philips 420 S(TEM).

The Philips 420 S(TEM) has the capability for quantitative and analytical microscopy. It was purchased in 1983 by investigators in Biological Structure on a BRSG shared instrumentation grant awarded by NIH. In addition, the Department has a Balzers 301 freeze-fracture unit and other major pieces of equipment (ultramicrotomes, ultracentrifuges, computing spectrophotometers, gamma and beta counters, etc.) which are maintained in departmental equipment rooms for shared use by departmental programs. All this equipment is available to and is extensively used by our postdoctoral fellows and graduate students who are active participants in departmental research programs. The equipment is maintained by service contracts the cost of which is shared by the principal users.

Maintenance

The Gammacell 40 will be installed in Room H-225 in the Department of Biological Structure. This room is adjacent to a newly constructed Vivarium and is conveniently

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ATTACHMENT 1

FACULTY RESUMÉS

Principal Investigator: Cornelius Rosse, M.D., D.Sc.
Professor and Chairman

Co-Investigators:
L.M. Ching, Ph.D.
Research Assistant Professor
A.G. Farr, Ph.D.
Assistant Professor
B.L. Hamilton, M.D., Ph.D.
Assistant Professor
M.Y. Lee, M.D.
Research Assistant Professor
L.A. Loeb, M.D., Ph.D.
Professor
S.B. Pollack, Ph.D.
Research Associate Professor
N.S. Wolf, D.V.M., Ph.D.
Associate Professor

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BIOGRAPHICAL SKETCH
CORNELIUS ROSSE

BORN: January 13, 1938; Csorna, Hungary

EDUCATION:

University of Bristol, England	B.Sc.	1961	Anatomy
University of Bristol, England	M.B.,Ch.B.	1964	Medicine
University of Bristol, England	M.D. (Ph.D.)	1974	Medicine
University of Bristol, England	D.Sc.	1983	Medicine

APPOINTMENTS:

1981-present	Professor and Chairman, Department of Biological Structure, University of Washington School of Medicine
1975-1981	Professor, Department of Biological structure, University of Washington School of Medicine
1974-1975	Visiting Professor, Department of Anatomy, Royal College of Surgeons, University of London, Postgraduate School
1970-1975	Associate Professor, Biological Structure, University of Washington School of Medicine
1967-1970	Assistant Professor, Biological Structure, University of Washington School of Medicine
1965-1967	Demonstrator in Anatomy, University of Bristol, England
1965	House Physician to J. E. Cates, Royal Infirmary, University of Bristol, England
1964-1965	House Surgeon to Professors R. Milner-Walker and A. G. Riddell, Royal Infirmary, University of Bristol, England

REPRESENTATIVE PUBLICATIONS

Pollack, S. B. and C. Rosse 1984 Production of natural killer cells in bone marrow. In Proc. 10th RES Congress, S. Reichard (Ed.) (Submitted)

Ching, L. M., A. G. Farr, S. Hosier, S. K. Anderson, and C. Rosse 1984 Myeloid-lymphoid cell interactions in the generation of cytotoxic T lymphocyte precursors in colonies grown in vitro. (Submitted)

Ching, L. M., A. G. Farr, S. Hosier and C. Rosse 1984 The ontogeny of a post-thymic precursor T-cell which generates cytotoxic T lymphocyte precursor cells in colonies grown in vitro. (Submitted)

Hollinshead, W. H. and C. Rosse 1985 Textbook of Anatomy. 4th ed., Harper and Row, Publishers, New York (In Press)

Rosse, C. and D. K. Clawson 1980 The Musculoskeletal System in Health and Disease. Harper and Row, Publishers, New York.

Dorshkind, K. and C. Rosse 1982 Physical, biological and phenotypic properties of natural regulatory cells in murine bone marrow. Am. J. Anat. 164: 1.

Lee, M. Y. and C. Rosse 1982 Depletion of lymphocye subpopulations in primary and secondary lymphoid organs of mice by a transplanted granulocytosis inducing mammary carcinoma. Cancer Res. 42: 1255.

Wolf, N. and C. Rosse 1982 Distribution and identity of the earliest proliferating progeny of colony-forming cells in regenerating murine spleen and bone marrow. Am. J. Anat. 163: 131.

Scuderi, P. and C. Rosse 1981 tumor neutralization by immune in vitro sensitized splenocytes depends on an interaction with bone marrow derived cells. Int. J. Cancer 27: 213.

Landreth, K., C. Rosse and J. Clagett 1981 The myelogenous phase in the production and maturation of primary B lymphocytes in the mouse. J. Immunol. 127: 2027.

Klimpel, G. R., K. Dorshkind, K. D. Klimpel and C. Rosse 1981 Bone marrow cytotoxic precursor T cells: alloantigen induced cytotoxic T cell responses by murine bone marrow cells in vitro. Cell. Immunol. 61: 154.

Rosse, C. 1980 Perspectives of lymphocyte production and cellular traffic in bone marrow. In The Handbook of Cancer Immunology. H. Waters, Ed., Garland ATPM Press, Washington, D.C., Vol. 6, p. 250.

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BIOGRAPHICAL SKETCH

LAI-MING CHING

BORN: January 12, 1950

EDUCATION:

University of Auckland, Auckland, NZ	B.Sc.	1973	Chemistry
University of Auckland, Auckland, NZ	M.Sc.	1975	Cell Biology
University of Auckland, Auckland, NZ	Ph.D.	1978	Immunology
Ontario Cancer Inst., Toronto, Canada	Postdoc.	1980	Immunology

APPOINTMENTS:

1984-present	Research Assistant Professor, Dept. Biological Structure, Univ. Washington School of Medicine, Seattle
1981-1983	Research Associate, Dept. Biological Structure, Univ. Washington, Seattle
1978-1980	Postdoctoral Research Fellow, Ontario Cancer Inst., Toronto
1973-1977	Laboratory Demonstrator in Cell Biology, Univ. Auckland

REPRESENTATIVE PUBLICATIONS

Ching, L. M., A. G. Farr, S. Hosier, C. Rosse and S. K. Anderson 1984 Myeloid-lymphoid cell interactions in the generation of cytotoxic T lymphocyte precursors in colonies grown *in vitro*. Submitted.

Ching, L. M., A. G. Farr, C. Rosse and S. Hosier 1984 The ontogeny of a post-thymic precursor T-cell which generates cytotoxic T lymphocyte precursor cells in colonies grown *in vitro*. Submitted.

Ching, L.-M. and R. G. Miller 1982 Development of cytotoxic T lymphocyte precursor cells in T cell colonies grown *in vitro*. J. Immunol. 129: 2345-2351.

Miller, R. G., S. Muraoka and L.-M. Ching 1982 Differentiation of functional T-cell subsets from immature precursors in murine T-cell colonies. Behring Inst. Mitt., No. 70, 9-13.

Ching, L.-M., S. Muraoka and R. G. Miller 1982 Differentiation of T cells from immature precursors in murine T-cell colonies. J. Immunol. 127: 2156-2163.

Ching, L.-M. and R. G. Miller 1981 Generation of cytotoxic T lymphocyte precursor cells in T cell colonies grown *in vitro*. Nature 289: 802-803.

Ching, L.-M. and R. G. Miller 1980 Characterization of *in vitro* T-lymphocyte colonies from normal mouse spleen cells: Colonies containing cytotoxic lymphocyte precursors. J. Immunol. 124: 696-701.

Ching, L.-M. and J. Marbrook 1979 The clonal analysis of cytotoxic lymphocytes against 2,4,6-trinitrophenyl (TNP)-modified cells. Eur. J. Immunol. 9: 22-27.

Ching, L.-M. 1978 Analysis of clones of cytotoxic lymphocytes. Ph.D. Thesis.

Ching, L.-M., K. Z. Walker and J. Marbrook 1977 Spontaneous clones of cytotoxic T cells in culture. III. Discriminatory lysis of pairs of syngeneic blasts induced by different mitogens. Eur. J. Immunol. 7: 850-854.

Ching L.-M., K. Z. Walker and J. Marbrook 1977 Clones of cytotoxic lymphocytes in culture: the difference in specificity between stimulated and non-stimulated cytotoxic lymphocytes. Eur. J. Immunol. 7: 846-850.

Ching, L.-M., J. Marbrook and K. Z. Walker 1977 Spontaneous clones of cytotoxic T cells in culture. II. Specificity of the response. Cell. Immunol. 31: 293-301.

Ching, L.-M., J. Marbrook and K. Z. Walker 1977 Spontaneous clones of cytotoxic T cells in culture. I. Characteristics of the response. Cell. Immunol. 31: 284-292.

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BIOGRAPHICAL SKETCH

ANDREW G. FARR

BORN: February 22, 1948

EDUCATION:

Univ. Colorado, Boulder	B.A.	1971	Biology
Univ. Chicago, Chicago	Ph.D.	1975	Anatomy
Harvard Med. Sch., Boston	Postdoc.	1979	Immunology
Nat. Jewish Hosp. & Res. Center, Denver	Postdoc.	1980	Immunology

APPOINTMENTS:

1984-present	Affiliate Member, Diabetes Research Center, Univ. Washington
1982-present	Assistant Professor, Department of Biological Structure, Univ. Washington
1981-1982	Member, Basel Inst. for Immunology, Basel, Switzerland
1980-1981	Research Associate, Dept. Pathology, Univ. Colorado Health Science Center, Denver
1979-1980	Postdoctoral Fellow, Dept. Medicine, National Jewish Hosp. and Research Center, Denver
1975-1979	Postdoctoral Fellow, Dept. Pathology, Harvard Med. Sch. Boston
1972-1975	Laboratory Demonstrator in Histology, Univ. Chicago

REPRESENTATIVE PUBLICATIONS:

Farr, A. G. and S. K. Anderson 1984 In situ ultrastructural demonstration of cells bearing Ia antigens in the murine pancreas. Diabetes (Submitted)

Ching, L. M., Hosier, S., Anderson, S., Rosse, C. and Farr, A. G. 1984 Myeloid lymphoid cell interactions in the generation of cytotoxic T lymphocyte precursors in colonies grown in vitro. Blood (Submitted)

Ching, L. M., Farr, A. G., Rosse, C., and Hosier, S. 1984 The ontogeny of a post-thymic precursor T-cell which generates cytotoxic T lymphocyte precursor cells in colonies grown in vitro. Eur. J. Immunol. (Submitted)

Farr, A. G. and Sidman, C. L. 1984 Reduced expression of Ia antigens by stromal cells of aged mice. J. Immunol. 133: 98.

Farr, A. G. and Nakane, P. K. 1983 Cells bearing Ia antigens in the murine thymus: An ultrastructural study. Am. J. Pathol. 111: 88.

Farr, A. G. and Nakane, P.K. 1981 Immunohistochemistry with enzyme labeled antibodies: A brief review. J. Immunol. Methods 47: 129.

Unanue, E. R., Farr, A. G. and Kiely, J. M. 1980 Multiple interactions between Listeria-immune T lymphocytes and macrophages: regulation by the major histocompatibility complex. In: Mononuclear Phagocytes: Functional Aspects, R. van Furth, Ed., Martinus Nijhoff Publishers, The Hague.

DeBruyn, P. P. H. and Farr, A. G. 1980 Lymphocyte-reticuloendothelial system interactions and their fine structural correlates. In: The Reticuloendothelial System: A Comprehensive Treatise. M. Escobar, H. Friedman and S. Reichard, Eds., Plenum Press, New York, 1980.

Farr, A. G., Cho, Y., and DeBruyn, P. P. H. 1980 The structure of the sinus wall of the lymph node relative to its endocytic properties and transmural passage of cells. Am. J. Anat. 157: 265-284.

Farr, A. G., Wechter, W. J., Kiely, J. M. and Unanue, E. R. 1979 Induction of cytocidal macrophages following in vitro interactions between Listeria immune T cells and macrophages. J. Immunol. 122: 2405-2412.

Farr, A. G., Kiely, J. M. and Unanue, E. R. 1979 Macrophage-T-cell interactions involving Listeria monocytogenes. Role of the H-2 gene complex. J. Immunol. 122: 2395-2404.

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BIOGRAPHICAL SKETCH

BRIAN L. HAMILTON

BORN: April 2, 1947; Portland, Oregon

EDUCATION:

Central Washington State College	B.A.	1969	Zoology
University of Washington, Seattle	Ph.D.	1975	Pathology
University of Washington, Seattle	M.D.	1976	Medicine

APPOINTMENTS:

1982-present Assistant Professor, Department of Biological Structure, University of Washington, Seattle, Washington

1982-present Adjunct Assistant Professor, Dept. Pediatrics, University of Washington, Seattle, Washington

1981-1982 Research Assistant Professor, Dept. Biological Structure, Univ. Washington School of Medicine, Seattle,

1978-1981 Fellow in Pediatric Immunology, Children's Hospital Medical Center, Harvard Medical School, Boston, MA

1976-1978 Intern and Resident in Pediatrics, Children's Medical Center, Dallas, TX

REPRESENTATIVE PUBLICATIONS:

Hamilton, B. L. 1984 Absence of correlation between cytolytic T lymphocytes and lethal murine graft-versus-host disease in response to minor histocompatibility antigens. Transplantation 38: 357-360.

Hamilton, B. L. and Harris, D. 1984 Prevention of graft-versus-host disease using antibody to Thy-1: A role for complement in vivo. Transplantation (In Press).

Mauch, P., Lipton, J. M., Hamilton, B. L., Obbugy, J., Kudisch, M., Nathan, D. G. and Hellman, S. 1984 Acute graft-versus-host disease: Modification with allogeneic cultured donor cells. Blood 63: 1112-1119.

Mauch, P. J., Lipton, J. M., Hamilton, B. L., Obbagy, J., Nathan, D. G. and Hellman, S. 1984 Reduction of lethal graft-versus-host disease: Transplantation of murine cultured cells across minor histocompatibility differences. Blood (In Press).

Hamilton, B. L. and R. Parkman 1983 Acute and chronic graft-versus-host disease induced by minor histocompatibility antigens in mice. Transplantation. 36: 150-155.

Kudisch, M., B. L. Hamilton and J. M. Lipton 1983 Early detection of hematopoietic engraftment in murine bone marrow transplantation utilizing hemoglobin electrophoretic differences. Transplantation 35: 515-518.

Duprez, V., Hamilton, B. L. and Burakoff, S. J. 1982 The generation of cytotoxic T lymphocytes in thymectomized irradiated and bone marrow-reconstituted mice. J. Exp. Med. 156: 844-859.

1078568

BIOGRAPHICAL SKETCH

MINAKO YOSHIOKA LEE

BORN: April 17, 1935, Tokyo, Japan
Japanese (Permanent U.S. visa)

EDUCATION: Jiyu-Gakuen Junior, Senior High School and College, Tokyo, 1957
Tokyo Women's Medical College, Tokyo, Japan, M.D., 1963

APPOINTMENTS:

1979-present	Research Assistant Professor, Depts. Medicine and Biological Structure, Univ. Washington School of Medicine, Seattle
1977-1979	Acting Instructor, Division of Hematology, Dept. Medicine, Univ. Washington School of Medicine, Seattle
1975-1977	Research Fellow, Division of Hematology, Dept. Medicine, Univ. Washington School of Medicine, Seattle
1970-1972	Resident in Clinical Pathology, VA Hospital, Minneapolis,
1969-1970	Resident in Pathology, Northwestern Hospital, Minneapolis,
1967-1968	Resident in Pathology, Mayo Clinic, Rochester, Minnesota
1966-1967	Resident in Medicine, Northwestern Hospital, Mineapolis
1965-1966	Internship, Cook County Hospital, Chicago,
1964-1965	Resident in Medicine, U.S. Air Force Hospital, Tachikawa, Japan,
1963-1964	Internship, U.S. Air Force Hospital, Tachikawa, Japan

REPRESENTATIVE PUBLICATIONS: (From a total of 22)

Lau, K. H. W., Lee, M. Y., Linkhart, T. A., Mohan, S., Vermeiden, J. and Baylink, J. Hypercalcemia induced by a mouse CE mammary carcinoma is mediated by a humoral factor which stimulates local prostaglandin in bone. J. Biolog. Chemist (Submitted)

Lee, M. Y. and Lottsfeldt, J. L. 1984 Augmentation of neutrophilic granulocyte progenitors in the bone marrow of mice with tumor-induced neutrophilia: Cytochemical study of in vitro colonies. Blood 64: 499-506.

Lee, M. Y. and Wolf, N. S. 1984 Effect of a neutrophilia inducing tumor on hemopoietic stem cells in mice. Int. J. Cell Cloning 2: 185-199.

Lee, M. Y. and Baylink, D. 1983 Hypercalcemia, excessive bone resorption, and neutrophilia in mice bearing a mammary carcinoma. Proc. Soc. Exp. Biol. Med. 172: 424-429.

Lee, M. Y., Uvelli, D. A., Agodoa, L. C. Y., Scribner, B. H., Finch, C. A. and Babb, A. L. 1982 Clinical studies of a continuous extracorporeal cyanate treatment system for patients with sickle cell disease. J. Lab. Clin. Med. 100: 334-344.

Lee, M. Y. and Rosse, C. 1982 Depletion of lymphocyte subpopulations in primary and secondary lymphoid organs of mice by a transplanted granulocytosis-inducing mammary caricnoma. Cancer Res. 42: 1255-1260.

Pootrakul, P., Hungsprenges, S., Baylink, D., Thompson, E., English, E., Lee, M., Burnell, J. and Finch, C. 1981 Relationship between erythropoiesis and bone metabolism in thalassemia. New Engl. J. Med. 304: 1470-1473.

Lee, M. Y., Sperlin, A. and Dale, D. C. 1980 Distribution of granulocytopoietic committed stem cells in mice with tumor induced neutrophilia. Exp. Hematol. 8: 249-255.

Finch, C. A., Price, T. H. and Lee, M. Y. 1979 Neutrophil kinetics in man. In Granulocyte Physiology, Function and Dysfunction, an American Association of Blood Banks Publication, pp. 9-33.

Lee, M. Y. and Rosse, C. 1979 Replacement of fatty marrow by active granulocytopoietic bone marrow following transplantation of mammary carcinoma into mice. Anat. Rec. 195: 31-46.

Lee, M., Durch, S., Dale, D., and Finch, C. A. 1979 Kinetics of tumor-induced murine neutrophilia. Blood 53: 619-632.

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BIOGRAPHICAL SKETCH

LAWRENCE A. LOEB, M.D., PH.D.

BORN: December 25, 1936, Poughkeepsie, New York

EDUCATION:

College of the City of New York, N.Y.	B.S.	1957	
New York University-Bellevue Medical School Salk Scholarship in Medicine	M.D.	1961	
Stanford-Palo Alto Medical Center, CA	Intern.	1962	Medicine
National Cancer Institute, Bethesda, MD	Postdoc.	1964	
University of California, Berkeley, CA	Ph.D.	1967	Biochemistry

APPOINTMENTS:

1978-present	Professor, Department of Pathology, University of Washington School of Medicine, Seattle, WA
1978-present	Director, Joseph Gottstein Memorial Cancer Research Laboratory. University of Washington, Seattle, WA
1978-present	Adjunct Professor, Department of Biochemistry, University of Washington, Seattle, WA
1977-1978	Senior Member, Institute for Cancer Research, Fox Chase, Philadelphia, PA
1971-1977	Associate Member, Institute for Cancer Research, Fox Chase, Philadelphia, PA
1967-1971	Assistant Member, Institute for Cancer Research, Fox Chase, Philadelphia, PA
1972-1978	Associate Professor, Department of Pathology, University of Pennsylvania, Philadelphia, PA
1968-1971	Assistant Professor, Department of Pathology, University of Pennsylvania, Philadelphia, PA
1973-1978	Member, Graduate Group in Molecular Biology, University of Pennsylvania, Philadelphia, PA
1968-1978	Member, Graduate Group in Biology, University of Pennsylvania. Philadelphia, PA
1964-1967	Research Associate, Department of Zoology, University of California, Berkeley, Berkeley, CA
1962-1964	Research Associate, National Cancer Institute, Bethesda, MD

REPRESENTATIVE PUBLICATIONS

Loeb, L.A., J. Abbotts, P.K. Liu and J.R. Silber Fidelity of DNA synthesis in eucaryotic cells. U.C.L.A. Symposia (In Press).

Kunkel, T.A., R.M. Schaaper and L.A. Loeb Depurination-induced infidelity of deoxyribonucleic acid synthesis with purified deoxyribonucleic acid replication proteins in vitro. Biochemistry (In Press).

Silber, J.R. and L.A. Loeb The molecular basis of environmental mutagenesis. In Environmental Pathology. N.K. Mottet (Ed.). Oxford University Press, New York (In Press).

Kunkel, T.A., R.M. Schaaper, E. James and L.A. Loeb Infidelity of DNA replication as a basis of mutagenesis. Plenum Press, New York (In Press).

Monnat, R.J. and L.A. Loeb Molecular biology and malignancy: Series Introduction. Mechanisms of neoplastic transformation. Cancer Investigations (In Press).

Kunkel, T.A., E.A. James and L.A. Loeb 1983 The uses of ΦX174 amber mutants for studying the fidelity of DNA polymerases. In DNA Repair. A Laboratory Manual of Research Procedures. E.C. Friedberg and P.C. Hanawalt (Eds.). Marcel Dekker, New York, Vol. 2, Chapter 16, pp. 223-237.

Aizawa, S., L.A. Loeb and G.M. Martin 1983 Isolation and preliminary characterization of mouse teratocarcinoma cells resistant to aphidicolin and arabionfuranosyl cytosine. J. Cell. Physiol., 115: 9-14.

Shearman, C.W. and L.A. Loeb 1983 On the fidelity of DNA replication XIV: Specificity of nucleotide substitution by intercalating agents. J. Biol. Chem., 258: 4477-4484.

1078570

BIOGRAPHICAL SKETCH

SYLVIA BYRNE POLLACK

BORN: October 18, 1940, Ithaca, New York

EDUCATION:

Syracuse Univ., Syracuse NY	B.A.	1962	Zoology
Univ. Pennsylvania, Philadelphia PA	Ph.D.	1967	Develop. Biology

APPOINTMENTS:

1981-present	Research Associate Professor, Dept. Biological Structure, Univ. Washington School of Medicine, Seattle,
1979-1981	Associate Member, Fred Hutchinson Cancer Research Center, Seattle
1977-1981	Research Associate Professor, Dept. Microbiology and Immunology, Univ. Washington, Seattle
1975-1979	Assistant Member, Fred Hutchinson Cancer Research Center, Seattle
1973-1977	Research Assistant Professor, Dept. Microbiology and Immunology, Univ. Washington School of Medicine, Seattle
1968-1973	Research Associate, Dept. Microbiology, Univ. Washington School of Medicine, Seattle
1967-1968	Instructor, Dept. Anatomy, Women's Medical College of Pennsylvania, Philadelphia

REPRESENTATIVE PUBLICATIONS: (From a total of 46)

Pollack, S. B. and C. Rosse 1984 Production of natural killer cells in bone marrow. In The Reticuloendothelial System. S. Reichard, Ed. Alan R. Liss, Inc., New York. (In Press)

Robles, C. P., P. Pereira, P. Wortley and S. B. Pollack 1984 Regulation of B cell responses by NK cells. In Mechanism for Cytotoxicity by NK Cells. R. B. Herberman, Ed., Academic Press, New York. (In Press)

Pollack, S. B. 1984 Phenotype, functional heterogeneity and lineage of natural killer killer cells. In Immunobiology of Natural Killer Cells. E. Lotzova and R. B. Hererman, Eds., CRC Press (In Press)

Emmons, S. L. and S. B. Pollack 1984 Murine NK cell heterogeneity: Subpopulations of C57BL/6 splenic NK cells detected by NK-1.1 and NK-2.1 antiserum. Submitted.

Pollack, S. B. 1983 In vivo functions of NK cells. Survey and Synthesis of Pathology Research. 2: 93-106.

Brooks, C. G., R. C. Burton, S. B. Pollack and C. S. Henney 1983 The presence of NK alloantigens on cloned cytotoxic T lymphocytes. J. Immunol. 131: 1391-1395.

Pollack, S. B. and S. L. Emmons 1982 NK 2.1: An NK-associated antigen detected with NZB anti-BALB/c serum. J. Immunol. 129: 2277-2281.

Lotzova, E., C. A. Savary and S. B. Pollack 1983 Prevention of rejection of allogeneic bone marrow transplants by NK 1.1 antiserum. Transplantation, 35: 490-494.

Pollack, S. B. and L. A. Hallenbeck 1982 In vivo reduction of NK activity with anti-NK 1 serum: Direct evaluation of NK cells in tumor clearance. Int. J. Cancer 29: 203-206.

Tam, M. R., S. L. Emmons and S. B. Pollack 1980 Analysis and enrichment of murine natural killer cells with the fluorescence-activated cell sorter. J. Immunol.

Pollack, S. B. 1982 Direct evidence for anti-tumor activity by NK cells in vivo: Growth of B16 melanoma in anti-NK 1.1 treated mice. In NK Cells and Other Natural Effector Cells. R. B. Herberman, Ed., Academic Press, pp. 1347-1352. 124: 650-655.

Pollack, S. B., M. R. Tam, R. C. Nowinski and S. L. Emmons 1979 Presence of T cell-associated antigens on murine NK cells. J. Immunol. 123: 1818-1821.

1078511

BIOGRAPHICAL SKETCH
NORMAN S. WOLF

BORN: July 22, 1927

EDUCATION:

Jr. College of Kansas City, MO	None	1944-45	Pre-Vet Med
Univ. Missouri, Columbia, MO	None	1947-48	Pre-Vet Med
Kansas State Univ., Manhattan KS	B.S. & DVM	1953	Veterinary Med
Northwestern Univ., Chicago, IL	Ph.D.	1960	Exp. Pathol.

APPOINTMENTS:

1968-present	Assoc. Prof., Dept. Pathol., Univ. Wash., Sch. Med. Seattle
1970-present	Member, Radiological Sci. Group, Univ. Wash. Sch. Med. Seattle
1968-1972	Head, Teaching Div. Dept. Exper. Animal Medicine, Univ. Washington, Seattle
1962-1968	Res. Asst. Prof. Div. Exper. Biol., Baylor College Med. Houston, TX
1961-1962	Consultant, Pathology & Physiology Section, Biol. Div., Oak Ridge Natl. Lab, Oak Ridge, TN
1960-1961	Visiting Scientist, Lab, Pasteur de l'Institut de Radium Paris
1960-1963	Grad. Fellow, Dept. Pathology, Northwestern Univ. Chicago IL

REPRESENTATIVE PUBLICATIONS

Lee, M. Y. and N. S. Wolf 1984 Effect of a neutrophilia inducing tumor on hemopoietic stem cells in mice. Int. J. Cell Cloning 2: 185.

Wolf, N. S., J. R. MacMillan and G. V. Priestley 1984 Decline of colony forming units with serial bone marrow passages: intrinsic or extrinsic causation? Blood Cells 9: 515.

Wolf, N. S. 1983 The hematopoietic microenvironment: Current status. In: Frontiers in Experimental Hematology. U. Torelli et al., Eds. Serono, Rome, pp. 233-251.

Wolf, N. S., G. V. Priestley and L. E. Averill 1983 Depletion of reserve in the hemopoietic system: III. Factors affecting the serial transplantation of bone marrow. Exp. Hematol. 8: 762.

MacMillan, J. R. and N. S. Wolf 1982 Depletion of reserve in the hemopoietic system: II. Decline in CFUs self-renewal capacity following prolonged cell cycling. Stem Cells 2: 45.

Wolf, N. S. and R. Arora 1982 Depletion of reserve in the hemopoietic system: I. Self-replication by stromal cells related to chronologic age. Mech. Age. Develop. 20: 127.

Wolf, N. S. 1982 Dissecting the hematopoietic microenvironment. V. Limitations of repair following damage to the hematopoietic support stroma. Exp. Hematol. 10: 108.

Wolf, N. S. 1982 Dissecting the hematopoietic microenvironment. IV. Regeneration of splenic microstructure - prerequisites and chronology of reocnstruction. Exp. Hematol. 10: 98.

Wolf, N. S. and C. Rosse 1982 Distribution and identity of the earliest progeny of colony-forming cells in regenerating murine spleen and bone marrow. Am. J. Anat. 163: 131.

Wolf, N. S. 1981 Studies utilizing the $S1/S1^d$ mouse model. In: Proc. Conf. on Aplastic Anemia, A Stem Cell Disease. NIH Publ. 81-1008, pp. 217-225.

Nishimura, E. T., R. G. Beegle and N. S. Wolf 1981 Ultrastructural lesion in fetal heopoietic cells following ethanol administration to pregnant mice. Lab. Invest. 45: 342.

Wolf, N. S. 1978 Dissecting the hematopoietic microenvironment. II. The kinetics of the erythron of the $S1/S1^d$ mouse and the dual nature of its anemia. Cell Tissue Kinet. 11: 335.

Wolf, N. S. 1978 Dissecting the hematopoietic microenvironment. III. Evidence for a positive short range stimulus for cellular proliferation. Cell Tissue Kinet. 11: 335.

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ATTACHMENT 2

ASSURANCE OF COMPLIANCE

APPENDIX I

Description and Specifications of Gammacell 40

List of Gammacell 40 Purchasers in the U.S.

Irradiators at the University of Washington

Mechanism for Authorization of Use of Gammacell 40

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Atomic Energy of Canada Limited
Industrial Products

SPECIFICATION NO. 830379

Gammacell 40
Low Dose Rate Laboratory Irradiator

A. GENERAL

A.1 The Gammacell 40 is a fully self-contained research irradiator designed to provide uniform gamma irradiation of small animals and biological samples. No additional shielding requirements are necessary to safely operate the unit. A Cesium-137 double-encapsulated source is housed in each of two round sliding drawers, one above and one below the sample cavity. The shielding design ensures that external radiation fields will not exceed levels established by the International Commission on Radiological Protection (Publication No. 15) and the United States National Commission on Radiation Protection and Measurements.

A.2 Radiation Specifications

Source Capsules:— 2-Model C161 Type 8 doubly encapsulated Cesium 137 sources,
— 1800 Ci each ± 15%

Unit Capacity:— 4,000 Ci Cesium 137

Central Dose Rate:— 7,900 rad/h or 132 rad/min ± 15%

Uniformity:— over 30.5 cm (12.0 in) diameter x 10.2 cm (4.0 in) high volume centred in the sample cavity, the dose rate ratio in air is approximately 1: 1.08 (approximately ± 5%). Typical isodose curves are shown on page 3.

A.3 Licensing

When applying for the Radioactive Materials License, customer should apply for 4000 curies, (the capacity of the unit) to allow for the ± 15% source loading tolerance.

B. COMPONENT PARTS

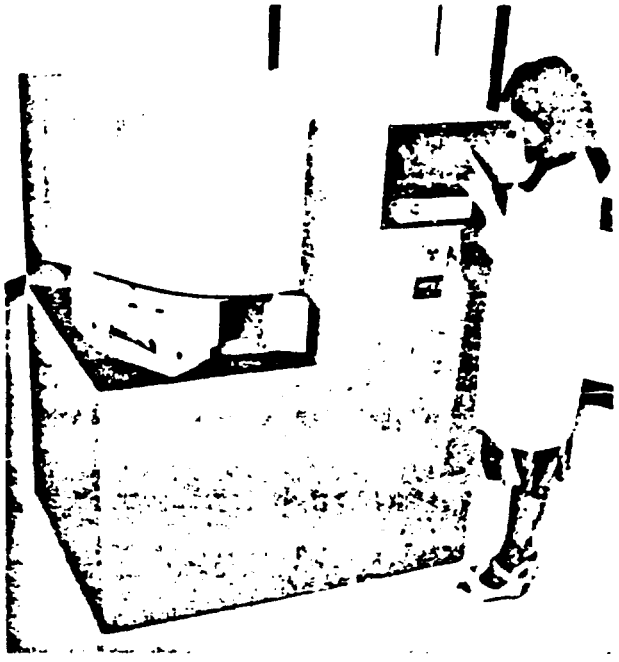
B.1 Radiation Shield

The radiation shield consists of steel-encased lead components arranged to completely surround the Cesium sources and sample cavity. The sources are housed in horizontally aligned, round, sliding drawers located above and below the sample cavity. The shield is designed for a nominal Cesium-137 capacity of 4,000 curies.

B.2 Sample Cavity

Dimensions of the irradiation sample cavity with a cylindrical ring fitted are:—

Depth:— 12.4 cm (4.9 in)
Diameter:— 33.1 cm (13.0 in)
Volume:— 10.60 cm³ (646 in³)



The cylindrical ring, open at both top and bottom ends, fits within the cavity. The ring is mounted to the hinged sample cavity door. Access to the samples, when the door is opened, can be achieved without reaching into the irradiation cavity.

A plastic sample tray with lid and support for use in the sample cavity is provided with the unit. The internal dimensions of the tray are 30.5 cm (12 in) diameter by 10.5 cm (4.13 in) deep. The sample tray has ventilation holes in its side which align with ventilation ports through the main shield.

B.3 Access Tubes

Three stainless steel access tubes 0.95 cm (0.375 in) inside diameter leading into the sample cavity are provided. Two of the tubes pass through the chamber door, the third tube passes through the fixed shield.

B.4 Source Drawers

The source drawers consist of round brass-encased, lead-filled, horizontal cylinders, 6.35 cm (2.5 in) diameter and 40.64 cm (16.0 in) long. The drawers are moved between the "Source On" and "Source Off" positions by means of pneumatic cylinders. Each drawer travels a total horizontal distance of 21.32 cm (8.0 in) in an elapsed time of

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less than 2 seconds. The timer starts when the drawers reach the "Source On" position.

B.5 Air Compressor

An air compressor, complete with reservoir tank, provides air for both drawer movement and sample cavity ventilation. The air system includes provision for supplying filtered, oil-free air to the sample cavity, and also for lubrication of the source-drawer cylinders. The compressor is driven by a ½ H.P. motor. (See Section E for power requirements.

B.6 Main Frame and Enclosure

The lower portion of the unit is enclosed in a sheet steel housing. The radiation shield is mounted on a steel frame with a skid-type base to facilitate easy movement during installation of the irradiator.

C. CONTROLS & SAFETY FEATURES

C.1 The control panel, located across the front of the irradiator, has the following controls:—

a) **Key Operated Master Switch** — controls power to the control panel and irradiator.

b) **"Manual/Auto" Mode Selector** — a split lens illuminated push-button switch. The upper half, for manual operation (without timer), illuminates white when the unit is in that mode. The lower half, for automatic operation (with timer), illuminates blue when the unit is in that mode. The alternate mode is selected by pushing the button.

c) **Digital Timer** — a five-digit readout timer; each digit is set by depressing a button mounted below the window. Readout range — 0.1 to 9999.9 minutes (0.6 seconds to 166.6 hours). Timer accuracy is determined by the electrical supply and is comparable to the accuracy of an electrical clock.

d) **"Source On"** — an illuminated push-button switch, controlling the movement of the source drawers to the irradiate position; illuminates red when sources are in their irradiate positions.

e) **"Source Off"** — an illuminated push-button switch, with a 2-section split lens, to control movement of the source drawer to the stored position. Upper and lower sections illuminate green when respective sources are in their stored positions.

f) **"Low Air/Timer On"** — a split lens, press-to-test type indicator lamp. The upper half illuminates red to indicate that air supply pressure has dropped below that required for reliable operation of the unit. The lower half illuminates to indicate timer on.

g) **"Chamber Air"** — an illuminated push-button switch to control the supply of forced air to the sample cavity; illuminates white when ventilation air supply is on.

C.2 Compressed Air Supply Controls

These controls are located beneath the sheet metal enclosure. Adjustment is not required for normal operation of the unit.

C.3 Safety Features

a) Electrical and mechanical interlocks are provided so that the sample cavity may be swung out for loading or unloading only when both source drawers are in the fully shielded position.

b) The source drawers are electrically and mechanically interlocked so that they cannot be moved from the safe position until the sample cavity door is closed.

c) The operator is not required to reach into the irradiation cavity to load or unload samples.

d) In the event of power failure or loss of air pressure, the source drawers move automatically to the fully shielded position and irradiation of samples is terminated until restarted by the operator. The sample cavity door cannot be opened for removal of samples while the power is off.

D. WEIGHTS AND DIMENSIONS

Total Weight:—	2,858 kg (6,300 lb)
Floor Loading:—	2,530 kg/m² (518 lb/ft²)
Projected Floor Area:—	1.13 m² (12.21 ft²)
Overall Width:—	92.4 cm (36.4 in)
Length:—	122.8 cm (48.4 in)
Height:—	146.5 cm (57.7 in)
Skid Clearances (clear cavity for lift truck):	
Width:—	79.7 cm (31.4 in)
Length:—	121.9 cm (48.0 in)
Height:—	17.8 cm (7.0 in)
Color of Unit:—	Seafoam Green with black control panel and kick panel.

E. ELECTRICAL POWER REQUIREMENTS

a) **Standard Unit**

This unit requires a power supply of 110/120 V, 60 Hz, single phase, 2 kVA. Fusing and wiring must be adequate for a ½ HP motor load. The circuit used to power the unit must have sufficient capacity to prevent release of relays in the unit caused by voltage drop when the compressor motor starts. An ON/OFF circuit switch in the immediate vicinity of the unit is recommended.

b) **Optional Power Supply**

The unit can be supplied to operate on 220/230 V, 50 Hz, single phase power supply. This option, if required, must be specified at the time of ordering.

NOTE: The type of power supply available (50Hz or 60 Hz) is to be specified at the time of ordering.

c) **Power Cable**

Where local electrical codes permit the unit may be plugged into a suitable wall receptacle. A 3m (10 ft) power cable with a three-prong plug is provided for this purpose.

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F. SHIPPING

The Gammacell 40, complete with Cesium-137 sources, is shipped without additional radiation shielding. Its design, construction and packaging comply with the Canadian and U.S.A. regulations covering transportation of radioactive materials. Shipping weight and dimensions are as follows:

Crated Gammacell 40:

Length:	213 cm (84 in)
Width:	122 cm (48 in)
Height:	173 cm (68 in)
Gross Weight:	3085 Kg (6,800 lb)

G. CERTIFICATION & DOCUMENTATION

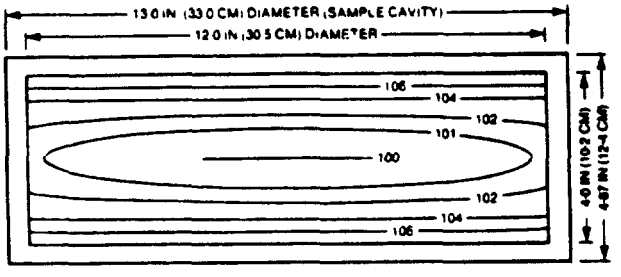
G.1 The dose rate at mid-point of the sample cavity is measured by ferrous chemical dosimetry to an accuracy of ± 3.5%. A Certificate of Calibration is supplied with each unit.

The Cesium source capsules are individually tested for leakage and contamination. A certificate describing the tests performed is supplied with each unit.

G.2 Current methods of Cesium-137 encapsulation do not permit a guarantee on the actual activity that will be shipped. It is anticipated that it will be within ± 15% of the nominal unit capacity stated in paragraph A.2 on Page 1.

G.3 A typical isodose pattern is shown below. Isodose measurements are not carried out on each individual unit. Numbers shown represent percentage of measured central dose rate.

DOSE DISTRIBUTION IN IRRADIATION CHAMBER



TWO VERTICALLY OPPOSED ^{137}Cs SOURCES OF EQUAL OUTPUT — GAMMACELL 40 ISODOSE CURVES VERTICAL PLANE

H. ACCESSORIES

H.1 Collimator (J10-059)

The collimator will permit partial irradiation of samples. Upper and lower lead shields reduce the central dose rate by approximately 95%. Six holes of 3 cm diameter are arranged in each shield to allow unattenuated beams of radiation from upper and lower sources to coincide at six points in the chamber.

H.2 Attenuators (set of 3) (J10-025)

With a set of attenuators the central dose rate can be reduced by 25% 50% or 75%

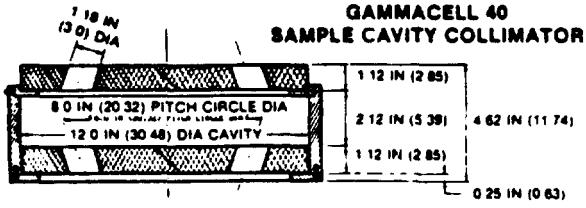
The set consists of steel-lead laminated disks separated by 3 posts.

H.3 Extra Sample Drawer Tray (J10-065)

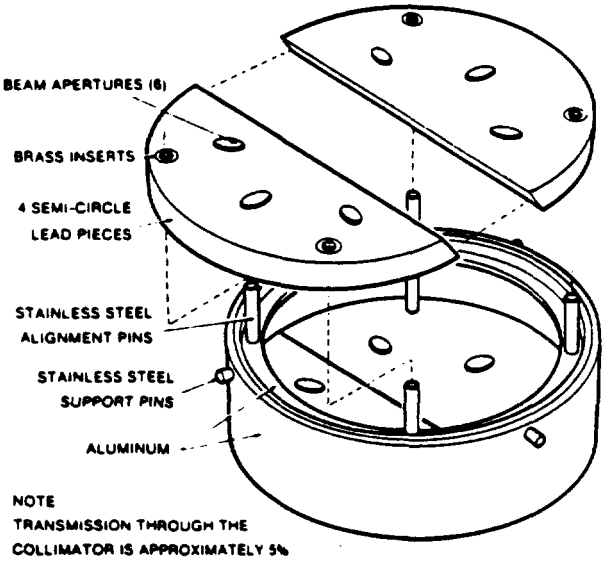
NOTE: RE WALL CLEARANCES:

Facing front of unit allow 6" between rear of unit and wall, 24" between right hand side of unit and wall, and 30" between left hand side of unit and wall to allow for servicing the unit and to allow the door to swing fully open.

GAMMACELL 40 SAMPLE CAVITY COLLIMATOR

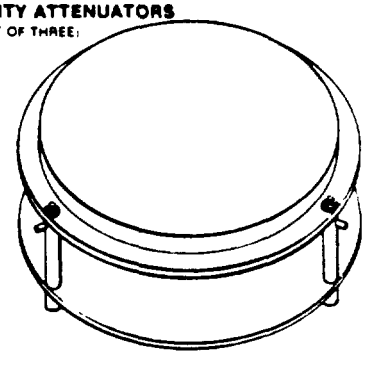


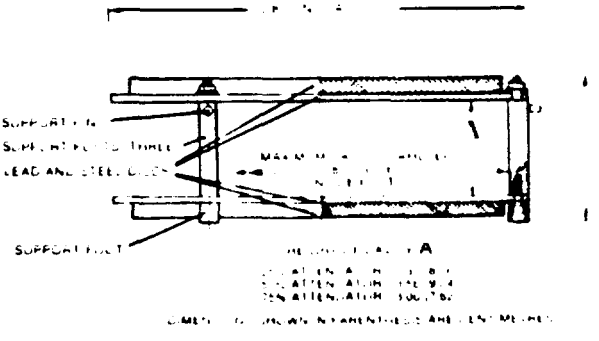
DIMENSIONS SHOWS IN PARENTHESIS ARE CENTIMETRES



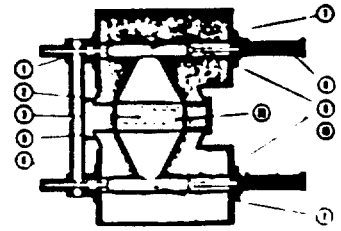
NOTE
TRANSMISSION THROUGH THE COLLIMATOR IS APPROXIMATELY 5%

GAMMACELL 40 SAMPLE CAVITY ATTENUATORS
(SET OF THREE)

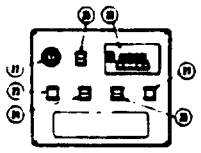




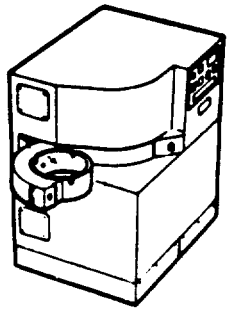
GAMMACELL 40
GENERAL DIMENSION LAYOUT



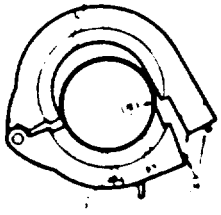
LEAD SHIELDING CROSS SECTION

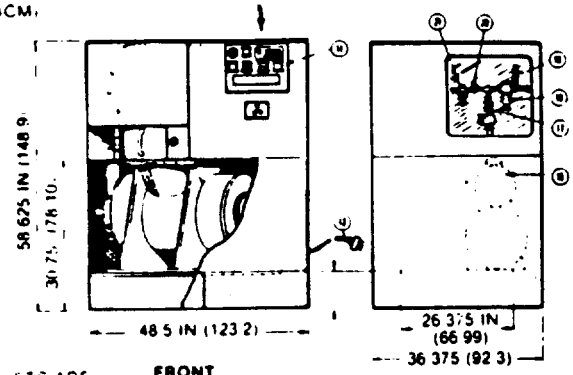


CONTROL PANEL



7 8 IN 9 UNC x 1 0 IN (2 54CM) TAPPED HOLED (3)





FRONT

SIDE

NOTE DIMENSIONS IN BRACKETS ARE CENTIMETERS

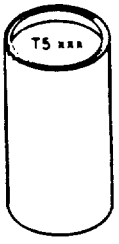
PARTS LIST

1 MECHANICAL INTERLOCK (UPPER)
2 ATTENUATOR (UPPER)
3 SAMPLE CAVITY
13 0 IN (33 0 CM) DIA x 4 87 IN (12 37 CM) DEEP
4 SAMPLE DRAWER TRAY (ABS PLASTIC)
5 ATTENUATOR (LOWER)
6 MECHANICAL INTERLOCK (LOWER)
7 SOURCE ON POSITION
8 SOURCE DRAWER CYLINDER (UPPER)
9 SOURCE OFF POSITION
10 SOURCE DRAWER CYLINDER (LOWER)
11 CONTROL PANEL
12 POWER CABLE - 10 FT (3M)
15 SAMPLE CHAMBER CAVITY RING
16 AIR COMPRESSOR & STORAGE TANK WITH GAUGE
17 PRESET VENTILATION VALVE
18 FILTER
19 PRESSURE SENSING SWITCH
20 AIR PRESSURE REGULATOR AND GUAGE
21 LUBRICATOR
22 POWER ON OFF KEY SWITCH
23 SOURCE ON PUSHBUTTON SWITCH
24 SOURCE OFF PUSHBUTTON SWITCH
25 MANUAL AUTO SELECTOR PUSHBUTTON SWITCH
26 DIGITAL TIMER
27 CHAMBER AIR ILLUMINATED PUSHBUTTON
28 TIMER ON LOW AIR INDICATOR LAMP

CAESIUM 137 CAPSULE ASSEMBLY

STANDARD SIZES

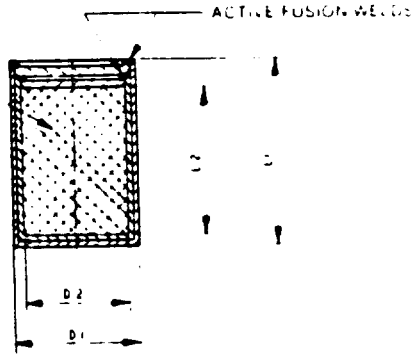
TYPE	D1 MAX OUTSIDE DIAMETER IN	D1 MAX OUTSIDE DIAMETER CM	D2 MAX ACTIVE DIAMETER IN	D2 MAX ACTIVE DIAMETER CM	L1 MAX OUTSIDE LENGTH IN	L1 MAX OUTSIDE LENGTH CM	L2 MAX ACTIVE LENGTH IN	L2 MAX ACTIVE LENGTH CM	NOMINAL CAPACITY IN CURIES	APPLICATION
1	1 377	3 396	1 250	3 175	2 665	6 819	2 30	5 84	3600	
2	1 106	2 809	0 984	2 500	1 925	4 889	1 52	3 82	1620	G9-095
3	1 237	3 142	1 125	2 858	1 680	4 267	1 28	3 07	1800	SQ DWR
4	1 381	3 508	1 250	3 175	1 680	4 267	1 29	3 07	2250	GC20 & SQ DWR
5	1 120	2 845	0 984	2 500	1 497	3 802	1 08	2 74	1170	
6	1 260	3 144	1 125	2 858	2 427	6 165	2 03	5 16	2810	GB 150
7	1 120	2 845	0 984	2 500	2 037	5 174	1 62	4 11	1740	
8	1 570	3 9nn	1 430	3 630	1 704	4 328	1 31	3 33	2940	GC 40



* When fully loaded inner capsule is inverted
When not fully loaded activity is centred vertically
** Capacities to be confirmed with isotope production

NOTES
1 Material Stainless Steel Type 316L
2 Wall Thickness 0 020 IN (0 053 CM)
3 Cap Thickness 0 085 IN (0 216 CM)
4 Engraving on Upper End Cap Serial Number

RADIOACTIVE MATERIAL CAESIUM 137



The specifications contained herein were in effect at the time of printing. Atomic Energy of Canada Limited has a policy of continuing development and reserves the right to discontinue models at any time or change specifications or designs without notice and without incurring obligation.

Atomic Energy of Canada Limited Industrial Products

P O Box 6300, Ottawa, Canada, K2A 3W3

Tel (613) 592-2790 • Cable Nemota • Telex 053-4162

Printed in Canada

1078578

LIST OF GAMMACELL-40 PURCHASERS IN THE U.S. 1982 January 29

University of Pennsylvania School of Medicine, PHILADELPHIA
St. Elizabeth Hospital Medical Center, BRIGHTON, Massachusetts
Scripps Clinic & Research Foundation, LA JOLLA, California
University of Texas S.W. Medical School, DALLAS, Texas
Massachusetts General Hospital, BOSTON, Massachusetts
Walter Reed Army Medical Center, WASHINGTON, D.C.
Veterans Administration Hospital, ST. LOUIS, Missouri
Sloan-Kettering Institute, RYE, New York
University of Connecticut School of Medicine, FARMINGTON
Harvard University Shields Warner Radiation Lab, BOSTON
Mt. Sinai Medical Center, MILWAUKEE, Wisconsin
Boston University Medical Centre, BOSTON, Massachusetts
Baltimore City Hospital Oncology Center, BALTIMORE, Maryland
Duke University, DURHAM, North Carolina
M.I.T. CAMBRIDGE, Massachusetts
Rush Presbyterian - St. Lukes Medical Center, CHICAGO, Illinois
Health Research In., Roswell Park Division, BUFFALO
Bollweevil Research Laboratories, MISSISSIPPI
East Carolina University Medical School, GREENVILLE, N.C.
Schering Corporation, BLOOMFIELD, New Jersey
Sidney Farber Cancer Institute, BOSTON, Massachusetts
John Hopkins Hospital, BALTIMORE, Maryland
Burroughs Wellcome Co., Research Triangle Park, NORTH CAROLINA
Northwestern University, Medical Micro Immunology Dept., CHICAGO, Illinois
National Jewish Hospital, DENVER, Colorado
Merck & Company, RAHWAY, New Jersey
Albert Einstein College of Medicine, BRONX, New York
U.S. Army Medical Materiel Agency, FORT DETRICK, Maryland
National Cancer Institute, BETHESDA, Maryland
Harvard Medical School, BOSTON, Massachusetts
University of Florida, Pathology Dept. J.H.M.H.C., GAINESVILLE, Florida
State University of New York at Stoneybrook, STONEYBROOK, New York
University of North Carolina Medical School, CHAPEL HILL, N.C.
Wayne State University School of Medicine, DETROIT, Michigan
National Institutes of Health, N.I.A.I.D., BETHESDA, Maryland
Jewish Hospital of St. Louis, ST. LOUIS, Missouri
National Institutes of Health, N.C.I., BETHESDA, Maryland
UCLA, LOS ANGELES, California

1078579

Emory University, Microbiology Department, ATLANTA, Georgia
National Institutes of Health, BETHESDA, Maryland
Columbia University Cancer Center, NEW YORK, New York
Bristol Laboratories, EAST SYRACUSE, New York
E.I. Dupont de Nemours and Company, WILMINGTON, Delaware
Uniformed Services University of the Health Sciences, BETHESDA, Maryland
Quidel Inc., La Jolla, California
Smith, Kline and French Laboratories, Philadelphia, Pennsylvania

1078580

University of Washington Correspondence

INTERDEPARTMENTAL

ENVIRONMENTAL HEALTH AND SAFETY
Radiation Safety Office GG-10

October 3, 1983

TO: Dr. Cornelius Rosse
Biological Structure

FROM: Michael J. O'Brien MJOB
Radiation Safety Officer GG-10

SUBJECT: Sealed Sources

As requested in your call, attached is a list of sealed sources similar to the Gammacell 40. We have included identification numbers, active or energy, dose rate and an estimation of the irradiation volume.

er
Enclosure

1078581

IRRADIATORS AT THE UNIVERSITY OF WASHINGTON:

	ID #	RADIO-NUCLIDE	ENERGY ACTIVITY	DOSE RATE AT 1 METER	IRRADIATION VOLUME
A.	In HEALTH SCIENCES				
1.	46	Cs-137	1509 Ci	500 R/hr	Open beam - small animal irradiator (Dept. of Radiobiology)
2.	41	Cs-137	1600 Ci	528 R/hr	Cell irradiator with petrie dish-size volume (Dept. of Medicine)
3.	Cabinet x-ray		50 KeV	100 R/sec	Low energy - only can do surface irradiation (Dept. of Genetics)
B.	CLINICAL UNITS IN UNIVERSITY HOSPITAL				
4.	Varian 6/100		6 MeV	0-200 R/min	For 10 cm^2 field - open beam
5.	Varian 20		15 MeV photons or 6, 9, 12, 20 MeV electrons	100-500 R/min in discreet stops	Open beam
C.	IN SCHOOLS OTHER THAN THOSE IN HEALTH SCIENCES				
6.	15	Co-60	10,000 Ci	120,000 R/hr	1-2 cubic feet under water (Dept. of Fisheries)
7.	29	Cs-137	1 Ci	330 mR/hr	TLD chip irradiator - cubic inches (Dept. of Ceramic Engineering)
8.	31	Cs-137	.35 Ci	120 mR/hr	Open beam (Dept. of Nuclear Engineering)
9.	32	Cs-137	.14 Ci	50 mR/hr	Open beam downward onto counter top (Dept. of Nuclear Engineering)
10.	61	Cs-137	30 Ci	10 mR/hr	Calibration source - open (Dept. of Nuclear Engineering)

*We are currently using instrument #1 which is referred to in the application.
*Instrument #2 is unavailable to us and is suitable only for irrradiating cells.
*All other instruments are inaccessible for biological research in the Health Sciences.

1078582

University of Washington Correspondence

INTERDEPARTMENTAL

ENVIRONMENTAL HEALTH AND SAFETY
Radiation Safety Office GG-10

October 3, 1983

TO: Cornelius Rosse, M.D., D.Sc.
Professor and Chairman
Department of Biological Structure SM-20

FROM: Michael J. O'Brien MJOB
Radiation Safety Officer
Radiation Safety Office GG-10

Any use of a sealed source at the University must be authorized by Radiation Safely. This should be a simple administrative action for the Gammacell 40. We will inspect the facility and make sure the personnel using it are qualified. The system will be placed in an inventory and leak test survey program which meets the requirements of our Washington State license. This is not a property control inventory program. Maintenance of mechanical systems will be your responsibility. To our knowledge, a similar instrument is not available in this area.

er

1078583

APPENDIX II

Graduate Students Benefiting from DOE-Sponsored

Research in the

Department of Biological Structure

1078584

8. Gong, Daniel M.S.
Thesis Supervisor: Dr. E.M. Eddy
Current Position
Unknown

9. Jaeger, Christine B. Ph.D.
Thesis Supervisor: Dr. Raymond Lund
Current Position
Postdoctoral Fellow
Dept. of Neurology
New York Hospital, Cornell Medical Center
New York, NY

10. Jen, Ling-Sun Ph.D.
Thesis Supervisor: Dr. Raymond Lund
Current Position
Assistant Professor
Dept. of Anatomy
Hong Kong

11. Kligman, Douglas Ph.D.
Thesis Supervisor: Dr. Mark A Nameroff
Current Position
Laboratory of Biochemical Genetics, Bldg. 36-1C06
National Institutes of Health
Bethesda, MD

12. Landreth, Kenneth Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Senior Research Scientist
Cancer Research Program
Oklahoma Medical Research Foundation
825 N.E. 13th
Oklahoma City, OK 73104

13. McKay, John Ph.D.
Thesis Supervisor: Dr. Mark A. Nameroff
Current Position
Post-doctoral Fellow
Fred Hutchinson Cancer Research Center
Seattle, WA 98104

14. Mulé, James J. Ph.D.
Thesis Supervisor: Dr. N.B. Everett
Current Position
D.C.T., N.C.I.
Dept. of Human & Health Sciences
Public Health Service
National Institutes of Health
Bethesda, MD 20205

1078585

15. Patton, Dorothy L. Ph.D.
Thesis Supervisor: Dr. Sheridan A Halbert
Current Position
Research Assistant Professor
Dept. of Obstetrics and Gynecology
University of Washington
Seattle, WA 98195

16. Press, Oliver, W. Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Instructor
Dept. of Medicine
University of Washington
Seattle, WA 98195

17. Quinn, LeBris Smith Ph.D.
Thesis Supervisor: Dr. Mark A. Nameroff
Current Position
Senior Fellow
Dept. of Pathology
University of Washington
Seattle, WA 98195

18. Scuderi, Phillip Jr. Ph.D.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Research Scientist
Carver Genetic Physics, Inc.
Seattle, WA 98105

19. Seiker, Larry C.
Thesis Supervisor: Dr. Lyle Jensen
Current Position
Research Assistant Professor
Dept. of Biological Structure
University of Washington
Seattle, WA 98195

20. Steiner, Verena M.S.
Thesis Supervisor: Dr. Cornelius Rosse
Current Position
Residency Program in Pathology
University of Vermont
Burlington, VT

1078586

B. Students Currently Benefiting from DOE-Sponsored Research in the Department of Biological Structure

1. Battaglia, David
2. Bohannon, Nancy Jones
3. Bramson, Rachel
4. Brewitt, Barbara A.
5. Davidson, David A.
6. Foster, Carolyn A.
7. Johnson, Lonnie
8. Kissinger, Charles R.
9. Liu, Hwan-Wun
10. Newman-Gage, Helen
11. Smith, Lynne
12. Stroeher, Virginia
13. Tupper, Joan C.
14. Vernon, Robert

1078587

UNIVERSITY OF WASHINGTON
SEATTLE, WASHINGTON 98195

The Graduate School
Grant and Contract Services

December 20, 1984

DOE University Research Instrumentation Program
Energy Program and Support Division
U. S. Department of Energy
Oak Ridge Operations
P.O. Box E
Oak Ridge, Tennessee 37831

8503073

Ladies and Gentlemen:

Enclosed are three proposals prepared by Professor Cornelius Rosse and Professor Karen A. Holbrook, Department of Biological Structure, and Research Associate Professor Barbara M. Hickey, School of Oceanography, requesting support under the "DOE University Research Instrumentation Program 1985."

Funding is requested as follows:
Dr. Rosse, $103,703 for the period of July 1, 1985 through June 30, 1986;
Dr. Holbrook, $160,710 for the period July 1, 1985 through June 30, 1986;
and Dr. Hickey, $278,855 for the period May 31, 1985 through April 30, 1986.

A proposal for a fourth investigator, who was selected under the guidelines, is planned to be forwarded soon.

To provide complete information requested on the cover page of the application, we have attached a list of all University of Washington D.O.E. awards active as of December 1, 1984. We hope this information is helpful as you review these proposals.

It is a pleasure to submit these proposals for your consideration.

Sincerely,

Donald W. Allen, Director
Grant and Contract Services

DWA/th
Enclosure

Please indicate
Principal Investigator's
name on all correspondence.

85-02-078

22 Administration Building, AD-24 / Telephone: (206) 543-4043

1078588

INSTITUTION:	UNIVERSITY OF WASHINGTON
PRINCIPAL INVESTIGATOR:	CORNELIUS ROSSE DEPARTMENT OF BIOLOGICAL STRUCTURE
AWARD AMOUNT:	$103,703
INSTRUMENT(S) REQUESTED:	GAMMACELL 40 LABORATORY IRRADIATOR
APPLICATION TITLE:	CELLULAR AND MOLECULAR BIOLOGY RESEARCH

The University of Washington proposes to purchase a Gammacell 40 Laboratory Irradiator to support seven energy-related biomedical and health related research programs in two departments.

Since 1965, faculty at the University of Washington have been involved in research on the health and environmental impacts of energy-related chemicals. The primary emphasis of DOE-sponsored research in this area has been on studying cellular defense mechanisms that are engaged in the recognition and neutralization of environmentally induced neoplasms. Currently, seven faculty members are involved in these research areas with DOE support and seventeen students have received their doctoral degrees as a result of DOE sponsored research. Fourteen graduate students are now working on M.S or Ph.D degrees in these areas.

The requested instrument will be specifically used in the following research areas: tumor growth mechanisms, genetic resistance or susceptability, experimental hematology and tumor immunobiology. The Gammacell 40 will have a significant impact on both the quantity and quality of research performed in experimental hematology, tumor immunobiology and molecular biology. The rate of progress in all the studies currently underway at the University of Washington in these areas has been limited by lack of a state-of-the-art irradiator for experimental work.

The University of Washington will cost share approximately 20 percent of the purchase price of the instrument.

1078589

STATEMENT OF WORK

Based on a series of external panel reviews and program staff discussions, this application has been selected for award under the terms of the FY 1985 University Research Instrumentation (URI) Program Announcement, DE-PS05-85OR21502. Details of the equipment to be purchased with this award are noted by reference in the attached application.

1078590

U.S. DEPARTMENT OF ENERGY

FEDERAL ASSISTANCE REPORTING CHECKLIST

FORM EIA-459A
(10/80)

FORM APPROVED
OMB NO. 1900-0127

1. Identification Number: 05-85ER75228.000	2. Program/Project Title: University Research Instrumentation Program

3. Recipient: University of Washington

4. Reporting Requirements:	Frequency	No. of Copies	Addressees
PROGRAM/PROJECT MANAGEMENT REPORTING			
[ ] Federal Assistance Milestone Plan			
[ ] Federal Assistance Budget Information Form			
[ ] Federal Assistance Management Summary Report			
[ ] Federal Assistance Program/Project Status Report			
[X] Financial Status Report, OMB Form 269	F	3	A, B
TECHNICAL INFORMATION REPORTING			
[X] Notice of Energy RD&D	O, X	3	A, B
[ ] Technical Progress Report			
[ ] Topical Report			
[X] Annual Technical Report *	Y, F	3	A, B

FREQUENCY CODES AND DUE DATES:

A - As Necessary; within 5 calendar days after events.
F - Final; 90 calendar days after the performance of the effort ends.
Q - Quarterly; within 30 days after end of calendar quarter or portion thereof.
O - One time after project starts; within 30 days after award.
X - Required with proposals or with the application or with significant planning changes.
Y - Yearly; 30 days after the end of program year. (Financial Status Reports 90 days).
S - Semiannually; within 30 days after end of program fiscal half year.

5. Special Instructions:

I. Distribution

Two (2) copies of all technical reports and one (1) copy of all financial reports are to be submitted to the DOE Project Officer (Addressee A - Block 11 of Notice of Financial Assistance Award (NFAA)).

One (1) copy of all technical reports, two (2) copies of financial status reports and all remaining reports are to be submitted to the DOE Contracting Officer (Addressee B - Block 12 of NFAA).

II. Annual Technical Reports

* Report should briefly describe the status and use of equipment, including research projects which have been undertaken as a result of the equipment being available, financial data on operation and maintenance expenses provided by Grantee, etc.

Annual reports will be required during the prescribed project period and will continue for at least two years following installation of the equipment.

6. Prepared by: (Signature and Date)	7. Reviewed by: (Signature and Date)
Donna Prokop 6/28/85	Richard Slipka

1078591

STATEMENT OF WORK

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[X] Financial Status Report, OMB Form 269	F	3	A, B
TECHNICAL INFORMATION REPORTING			
[X] Notice of Energy RD&D	O, X	3	A, B
[ ] Technical Progress Report			
[ ] Topical Report			
[X] Annual Technical Report *	Y, F	3	A, B

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Donna Prokop 6/28/85	Richard Slipka

1078591