Division of

CANCER TREATMENT

Volume I
October 1, 1982-September 30, 1983

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

National
Institutes of
Health

National
Cancer
Institute

National Cancer Institute

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1982 through September 30, 1983

CONTENTS

ANNUAL REPORT

DIVISION OF CANCER TREATMENT

October 1, 1982 through September 30, 1983

The Division of Cancer Treatment is that component of the National Cancer Institute responsible for the development and evaluation of new methods of cancer treatment. Research activities encompass all modes of therapy, including surgery, radiotherapy, chemotherapy, and immunotherapy, both individually and in combination. The Division's responsibilities are carried out through a variety of mechanisms. Investigator-initiated laboratory and clinical research is performed under the auspices of traditional research grants. Targeted activities of the Division, such as treatment development, are carried out through contracts and cooperative agreements. Complementary to these extramural programs are the activities, both laboratory and clinical, carried out by the DCT intramural staff.

Personnel and Organization

The Division currently consists of five operating programs, each headed by an Associate Director. A current organization chart is shown in Figure 1. Numerous personnel changes have occurred throughout the Division during the past year, and the major ones will be listed here according to program area.

A. Office of the Director (OD)

- o Dr. Arthur Levine left the DCT to become Scientific Director of the National Institute of Child Health and Human Development.
- o Dr. Stephen Sherwin joined the staff as a Special Assistant during the year, but left recently to enter private industry.

B. Clinical Oncology Program (COP)

- o Dr. Samuel Broder, who had been Acting Associate Director, was appointed to that position permanently.

C. Radiation Research Program (RRP)

- o Dr. Bruce Wachholz joined the staff as Chief, Low-Level Radiation Effects Branch.

D. Cancer Therapy Evaluation Program (CTEP)

- o Dr. Robert Wittes was appointed Associate Director.
- o Dr. William DeWys transferred to DRCCA and was replaced, on an acting basis, by Dr. Edwin Jacobs.
- o The Biometric Research Branch, headed by Dr. Richard Simon, was transferred from COP to CTEP.

E. Developmental Therapeutics Program (DTP)

- o Dr. Matthew Suffness, who had been Acting Chief, Natural Products Branch, was appointed permanently.

- o Dr. Charles Grieshaber was appointed Chief, Toxicology Branch.

F. Biological Response Modifiers Program (BRMP)

- o A new laboratory, the Laboratory of Molecular Immunoregulation, was established, headed by Dr. Joost Oppenheim, previously of NIDR.

Program Highlights

More detailed reports of the individual program areas within DCT will be presented later in this report. The following summaries describe selected highlights and activities of each program during Fiscal Year 1983. In addition, activities within the Office of the Director will be briefly summarized.

Office of the Director

International Treatment Research

The DCT activities in international treatment research are coordinated in the Office of the Director. During the past year they were administered by Dr. Arthur Levine and later Dr. Stephen Sherwin. Upon Dr. Sherwin's departure, these duties were assumed temporarily by Dr. Schepartz. International activities include liaison offices at the Institut Jules Bordet in Brussels and the Japanese Foundation for Cancer Research in Tokyo; international agreements with the USSR, France, Japan, Hungary, Federal Republic of Germany, Italy, People's Republic of China, Egypt, Poland and the Pan American Health Organization (PAHO); and close working relationships with investigators in the United Kingdom and other countries. These programs have led to exchanges of scientists, drugs and clinical protocols with valuable exchanges of information and a general enhancement of preclinical and clinical research in this country and abroad.

The agreement with Japan was especially active during the year. In October 1982, a Radiation Therapy Research Seminar was held in Chiba and Kyoto, Japan. Then, in November, the 8th Annual Program Review Meeting was held in Bethesda, at which time a workshop was held on the development and evaluation of new antitumor drugs in Phase I and II in the U.S. and Japan. Finally, in May 1983, a workshop was held in Honolulu on drug resistance. The other major bilateral agreement workshop was held under the U.S.-Italy agreement in Palermo during October 1982.

Scientific Information Branch (SIB)

The Scientific Information Branch consists of two sections, the Publications Section and the Literature Research Section, and three major areas of activities: dissemination of information through Cancer Treatment Reports,

Cancer Treatment Symposia, and PDQ; preparation of literature reviews and similar reports for DCT staff; and operation of the DCT library, primarily for staff located in the Landow Building.

Cancer Treatment Reports has served as a primary scientific journal since 1959. From January 1982 to June 30, 1983, the journal received 892 manuscripts, accepted 579, and published 18 issues totaling 2758 pages. It publishes full length manuscripts, brief communications, letters to the Editor, clinical trials summaries, special features, current controversies in cancer managememt, meeting reports, and mini-symposia. During this year, Dr. Robert Wittes, the Associate Director for the Cancer Therapy Evaluation Program, assumed the the position of Editor-in-Chief, succeeding Dr. John Macdonald.

The Literature Research Section serves the DCT by providing information from published literature in all areas of treatment, to assist staff in Decision Network review, IND filing, preparation of clinical brochures, and for other staff needs. During the past year more than 300 requests were filled, about 200 of which required both manual and computer searching of the literature.

Scientific Programs

Introduction

The Division of Cancer Treatment in the National Cancer Institute is responsible for discovering and implementing therapies for cancer. This program is pursued through both intramural and extramural research encompassing medical oncology and drug development, radiation therapy, surgery, and biological agents. Within this framework, there have been a number of important developments in the past year.

Clinical Studies

Within the intramural Clinical Oncology Program, an accelerated effort has been mounted to identify the cause and to test possible treatments for the Acquired Immune Deficiency Syndrome (AIDS) and its associated malignancies. A task force of NCI personnel has been organized through the Office of Director, NCI, and includes as its scientific leader, Dr. Robert Gallo of the Developmental Therapeutics Program and as clinical leader, Dr. Samuel Broder of the Clinical Oncology Program. This group of investigators is cooperating in the identification of the cause of AIDS and initial efforts have led to the isolation of HTLV isolates from 3 patients. In addition, clinical protocols have been instituted to test both chemotherapy and various biological therapies in AIDS. The response rate to combination chemotherapy at this point looks promising with 3 of 5 patients achieving clinical response. Two of 13 patients had objective responses with alpha interferon. There are plans in the coming year to test gamma interferon as well as interleukin-2 in this disease. Additional interest has focused on diseases caused by the human T-cell leukemia virus (HTLV). The T-cell lymphoma caused by HTLV is characterized by hypercalcemia, skin lesions and a rapid downhill course. It is seen predominately in blacks, particularly from the Carribean region. COP investigators have identified in one patient a cytotoxic T-cell line which recognizes HTLV-infected cells in an HLA-restricted manner.

Additional clinical protocols have provided interesting results. A trial of radiotherapy and surgery for primary treatment of breast cancer has now accrued 115 patients, although the results are not yet ready for analysis. A randomized study in patients with extremity soft tissue sarcoma has compared limited surgery and radiation versus radical surgery and has indicated no benefit for for the radical procedure. In addition, a study of the same patients treated with or without adjuvant therapy has shown a definite advantage for patients receiving adjuvant chemotherapy. In diffuse histiocytic lymphoma an improved response rate has been demonstrated with an intensive regimen employing high dose methotrexate. A new randomized trial comparing PROMACE plus MOPP versus PROMACE cytabom (an Ara-C containing regimen), has shown excellent results in both arms, with complete remission rates in the range of 75-90%. In childhood acute lymphocytic leukemia, high dose systemic methotrexate plus standard chemotherapy has proved to give equal results in terms of systemic remission rate and prevention of CNS relapse, as compared to standard therapy with intrathecal methotrexate and cranial irradiation. A new regimen employing VP-16 in addition to platinum, Velban and bleomycin has produced highly encouraging results in poor prognosis testicular cancer when compared with a concurrent PVB arm.

In the Biological Response Modifiers Program, Phase I clinical trials of recombinant alpha interferon have been completed and studies have been initiated at the Phase II level in breast and lymphoma, where clinical responses have been observed. In addition, a Phase I trial has been completed completed of thymosin fraction V and three responses have been observed in renal cell carcinoma. A Phase I trial of alpha I thymosin has also been completed, but no clinical responses were observed. Monoclonal antibody trials of T-101, an antibody to a T-cell surface antigen, have been instituted, as well as an antimelanoma antibody study. Results are too early to evaluate in both cases. The possibility of using adoptive immunotherapy with expanded tumor-specific T-cells has come to its initial clinical trial in the Surgery Branch of the Clinical Oncology Program.

Extramural Clinical Trials

New procedures have been instituted for accounting for drugs distributed by the Cancer Therapy Evaluation Program. Expanded on-site monitoring of clinical trials through the cooperative groups was instituted in the summer of 1982 and entered full swing in the current fiscal year. In addition, the list of satellite investigators has been completed and procedures have been instituted for strengthening the relationship between satellite and cooperative group members. An intensified, systematic review of informed consent forms associated with clinical protocols has also been instituted. In the Radiation Research area, the clinical unit at the NCI has moved to new quarters and expanded research has been initiated in tumor cell biology and radiosensitizers. In the Radiation Research Program, comprehensive plans have been developed for radiation research and for diagnostic imaging research. In the extramural grant program, protons and helium ions have been shown to produce a cure of uveal melanomas.

Preclinical Treatment Research

In basic pharmacologic studies, NCI investigators have shown that adriamycin forms a tight complex with iron, and undergoes an oxidation reduction cycle

which generates free radicals. These free radicals are capable of producing injury to DNA. The mechanism of resistance to adriamycin and other antibiotics antibiotics has been investigated in several model cell lines. It has been clearly demonstrated that cross-resistance can develop between unrelated antibiotics after exposure of cells to any one of several agents in culture, including adriamycin, vincristine, colchicine or actinomycin D. In some cases this resistance is reversible with calcium channel blocking agents. The determinants of response to antifolates have received increased attention in the past year. The importance of the process of polyglutamation, which produces methotrexate metabolites with prolonged retention and increased binding affinity for multiple intracellular sites, has been demonstrated in human tumors. In addition, gene amplification has been demonstrated in a cell line derived from a patient treated with methotrexate. This amplification occurred in double minute chromosomes and was unstable during continuous culture.

An interesting new agent, tiazofurin, which will enter clinical trial in the coming year, has been shown to inhibit IMP dehydrogenase through the formation of a metabolite which is an analog of NAD. A stable isotope technique has been developed for assessing an activity of de novo versus salvage pathways in tumor cells, and may be used for in vivo studies. Finally, models have been developed for evaluating nitrosourea toxicity to lung and kidney, and the potent effect of these derivatives on glutathione reductase has been demonstrated.

In the area of drug development and pharmacology, six new agents have entered clinical trial during the past year, including spiromustine, caracemide, tiazofurin, SR 2508, acodazole HCL and fludarabine phosphate. Extensive changes have taken place in the preclinical phases of the drug development process and these changes are under continuous evaluation. The human tumor stem cell assay (HTSCA) is being employed to select agents for clinical trial. Initial evaluation evaluation indicates that approximately 20% of selected agents negative in the standard P-388 in vivo screen may be positive in the HTSCA, and these compounds will be in more detail evaluated with the prospect of conducting a clinical trial. Agents identified by the expanded in vivo tumor panel will also undergo development with the aim of clinical evaluation.

In tumor biology research, attention has turned to onc genes in the past year and amplification of C-myc has been observed in large cell variants of small cell carcinoma. The polypeptide hormone, bombesin, appears to have a growth promoting function in small cell carcinoma, in that bombesin antibodies can abolish or restrict tumor growth. The process of hormonal control of cell proliferation has been clarified further by the finding that nuclear estrogen receptor complexes with its ligand and undergoes transformation in the nucleus with major changes in sedimentation co-efficient and molecular size.

The possibility of association of these complexes with a new intranuclear protein is suggested. The factors which promote cell traverse through the cell cycle have been additionally clarified with the isolation of two new histone variants which appear during the G_0 phase of the cell cycle.

In other laboratory investigations, the cells which are responsible for natural killer activity have been identified further and these appear to be large granular lymphocytes, the granules of which have cytotoxic activity against tumor cells in vitro. Interleukin-2 has been demonstrated to increase natural killer

cell activity. In addition, macrophage activating factor encapsulated in liposomes has been shown to reduce pulmonary metastases in rodents and is progressing toward clinical evaluation.

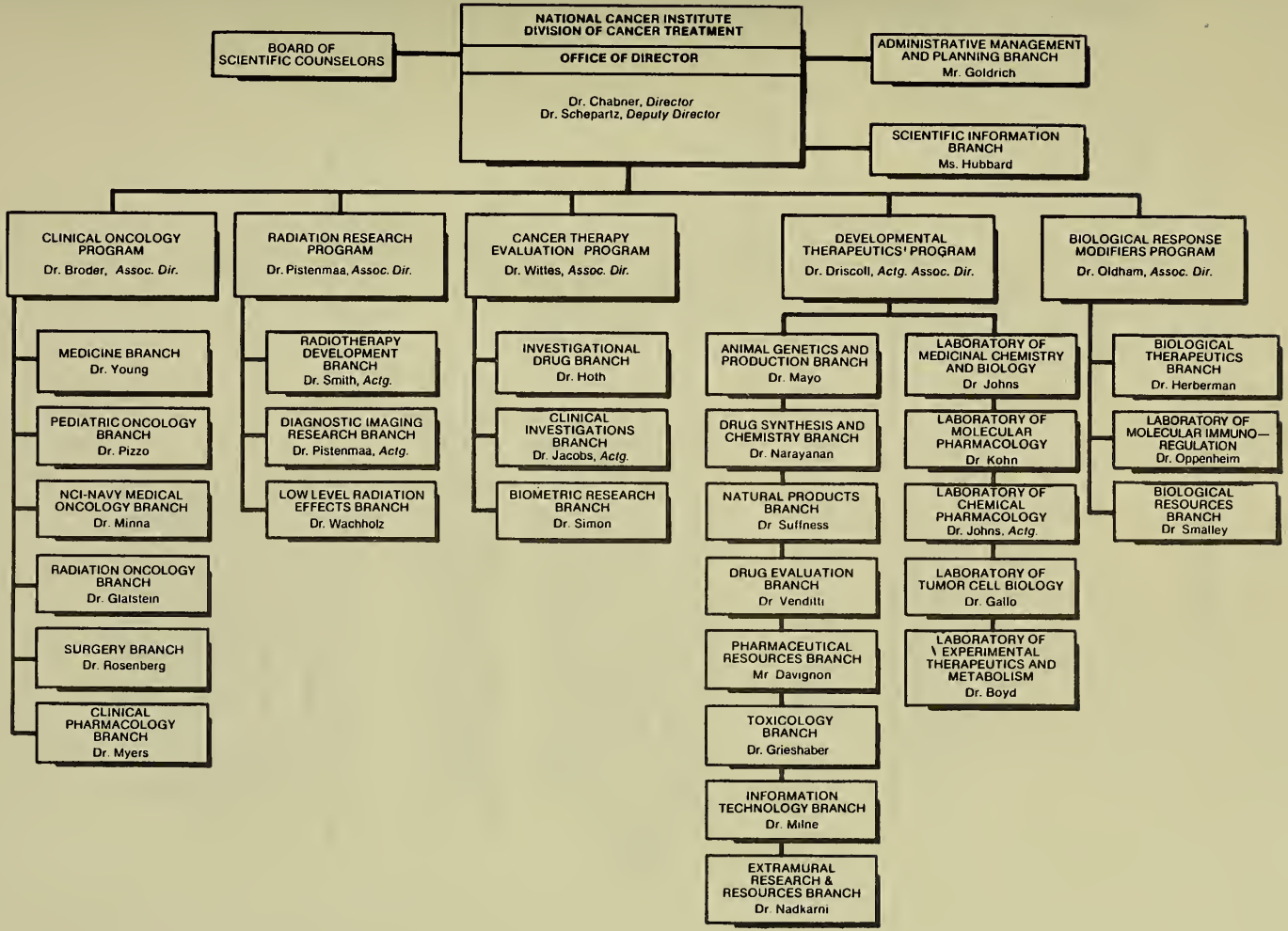
In summary, the clinical and laboratory programs supported by the Division of Cancer treatment have identified HTLV as a possible causative agent in patients with AIDS and the clinical features of HTLV-related T-cell lymphoma have been elucidated. Important new trials have been initiated for a number of biological agents as well as new drugs, and the mechanism of action and resistance of established agents such as methotrexate and adriamycin have been further clarified.

Publications

Chabner, B.A., Clendeninn, N., and Curt, G.: New clinical perspectives regarding drug development. Proc. 13th International Cancer Congress, Seattle, 1982. (In Press)

Schepartz, S.A.: Orphan Products Activities of the National Institutes of Health. In Brewer, G.J. (Ed.): Progress in Clinical and Biological Research, Vol. 127: Orphan Drugs & Orphan Diseases: Clinical Realities and Public Policy. A.R. Liss, Inc., New York, 1983, pp. 173-186.

Figure 1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Division of Cancer Treatment Stratification

Code	Description
PDD	Preclinical Drug Development Program -
1XX	Stage I - Acquisition of Materials
2A1	Stage II - Basic Screen - Determination of anti-tumor activity of new agents.
2B1	- Develop acceptable experimental formulation.
2B2	- Verification screen - Detailed evaluation of new agents (dose, route and schedule dependency).
2B3	- Procurement of sufficient amounts of new agents for preclinical studies.
3X1	Stage III - Toxicology and pharmacology in animals.
3X2	- Production and formulation for clinical trials.
PBR	Preclinical Basic Research -
PBS	Cellular/Subcellular Studies - Includes biochemistry, biological response modifiers, blood products, cell biology, cell kinetics, therapeutic nutrition, immunobiology, markers, molecular biology, radiobiology, transfusion research, hyperthermia, combined modalities, radiation modifiers, radiation immunology, radiation physics, comparative pharmacology, experimental therapy, mechanism of drug action, synthetic and natural products, and data processing.
PTS	Treatment Studies - Independent treatments, combined modality therapy, radiation physics, radiation equipment development, nuclear medicine and data processing.
CTR	Clinical Trials Research -
CT1	Phase I Clinical Trials* - Initial clinical evaluation of new drugs, clinical pharmacology.
CT2	Phase II Clinical Trials* - Allocation for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens have highest priority for initial efficacy evaluation.

CT3 Phase III Clinical Trials* - Allocation for specific disease-oriented resources to study whatever chemotherapy or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.

CT4 Phase IV Clinical Trials* - Allocation for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.

CT5 Statistics, Data Processing, and Other Clinical Trials Research

CSR Clinical Trials Supportive Research - Includes special pharmacology/toxicology, cell kinetics, markers, blood products, transfusion research, protected environment, hyperthermia, nutrition, statistics, and data processing.

MGT Program Management - Includes administration, dissemination of information to the medical and scientific community.

*Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES FOR FY83

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	24,395,726	61.01
STAGE I	6,205,600	15.52
1XX ACQUISITION OF MATERIALS		
STAGE IIA	6,161,663	15.41
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	5,435,826	13.59
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	180,818	0.45
2B2 DETAILED EVALUATION OF NEW AGENTS	2,356,833	5.89
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	2,898,175	7.25
STAGE III	6,592,637	16.49
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	2,447,579	6.12
3X2 PROD. & FORM. FOR CLINICAL TRIALS	4,145,058	10.37
PBR PRECLINICAL BASIC RESEARCH	6,103,753	15.26
PBS CELLULAR/SUBCELLULAR STUDIES	4,581,249	11.46
PTS TREATMENT STUDIES	1,522,504	3.81
CTR CLINICAL TRIALS RESEARCH	6,941,029	17.36
CT1 PHASE I CLINICAL TRIALS	1,733,623	4.34
CT2 PHASE II CLINICAL TRIALS	943,325	2.36
CT3 PHASE III CLINICAL TRIALS	1,292,181	3.23
CT5 OTHER CLINICAL TRIALS RESEARCH	2,971,900	7.43
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	1,842,043	4.61
MGT PROGRAM MANAGEMENT	706,164	1.77
TOTAL	39,988,715	100.00

463 CONTRACTS. NOT INCLUDED ARE ABOUT 9 SCHEDULE A CONTRACTS ($3,000,000) AND 47 SCHEDULE B CONTRACTS ($9,000,000). ALSO NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (ABOUT $2,000,000).

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	ALABAMA, UNIVERSITY OF	N CM0735515	14,802
D.T.P.	ALABAMA, UNIVERSITY OF	N CM0735516	13,325
D.T.P.	ALABAMA, UNIVERSITY OF	N CM0735517	10,657
D.T.P.	ALABAMA, UNIVERSITY OF	N CM0735518	9,437
D.T.P.	ALABAMA, UNIVERSITY OF	N CM2757100	138,502
A.P.	ALABAMA, UNIVERSITY OF	N CP9561600B	3,000
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	60,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	101,250
D.T.P.	BIOTECH RESEARCH LABORATORIES, INC.	N CM3755800	37,345
D.T.P.	BRISTOL LABORATORIES	N CM3755600	484,861
A.P.	CHARLES RIVER BREEDING LABS.	N CM1749800	9,864
A.P.	CHARLES RIVER BREEDING LABS.	N CM5059800	7,996
A.P.	CHARLES RIVER BREEDING LABS.	N CM8721200	16,000
A.P.	CHARLES RIVER BREEDING LABS.	N CM9016300	9,964
A.P.	CHARLES RIVER BREEDING LABS.	N CM9722900	76,460
D.T.P.	CHEMICAL ABSTRACTS SERVICE	N CM4372200	323,000
D.T.P.	ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900	74,619
D.T.P.	FLOW LABORATORIES, INC.	N CM2750500	270,391
D.T.P.	GEORGIA INSTITUTE OF TECHNOLOGY	N CM2751700	145,028
A.P.	HARLAN INDUSTRIES	N CM5059100	4,764
A.P.	HARLAN SPRAGUE DAWLEY, INC.	N CM2391100	380,681
BRMP	HARLAN SPRAGUE DAWLEY, INC.	N CM23911001	140,000
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	104,999
D.T.P.	ILLINOIS, UNIVERSITY OF	N CM3751300	105,769

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	INSTITUTE OF CANCER RESEARCH	N CM4373600	25,000
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	1,600
A.P.	KING ANIMAL LABORATORY	N CM1749900	5,848
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM5057700	6,388
BRMP	LITTON BIONETICS, INC.	N CM1580800	98,500
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	34,994
A.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700B	2,500
BRMP	MELOY LABORATORIES, INC.	N CM1575700	55,500
BRMP	MELOY LABORATORIES, INC.	N CM1581301	270,000
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N CM5700900	159,600
A.P.	MICROBIOLOGICAL ASSOCIATES	N CM9724600	63,991
A.P.	MISSOURI, UNIVERSITY OF	N CM2753400	13,246
A.P.	MISSOURI, UNIVERSITY OF	N CM8715700	33,501
A.P.	MURPHY BREEDING LABS., INC.	N CM5057900A	6,538
A.P.	NORTHWESTERN UNIVERSITY	N CM1736300	5,991
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000	30,396
D.T.P.	POLYSCIENCES, INC.	N CM0730000	45,000
D.T.P.	POLYSCIENCES, INC.	N CM3755700	392,488
A.P.	PROGRAM RESOURCES, INC.	N C02391000P	90,000
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM0735206	886
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM0735213	16,004
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM0735214	17,914
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM0735215	22,051
A.P.	SIMONSEN LABORATORIES	N CM5057800	7,974

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
A.P.	SIMONSEN LABORATORIES	N CM9724700	86,409
D.T.P.	SISA, INC.	N CM0735406	2,340
D.T.P.	SISA, INC.	N CM0735407	6,547
D.T.P.	SISA, INC.	N CM0735408	17,809
D.T.P.	SISA, INC.	N CM0735409	13,318
D.T.P.	SMALL BUSINESS ADMINISTRATION	N CM1740000	68,250
ISDT	SMALL BUSINESS ADMINISTRATION	N CM3760900	170,867
A.P.	SOUTHERN ANIMAL FARMS	N CM5059900	6,188
A.P.	SOUTHERN ANIMAL FARMS	N CM9724500	36,566
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM0726012	2,314
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM0726013	1,751
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM0726014	29,222
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM0726015	19,057
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	16,458
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N CM0735606	17,088
D.T.P.	SOUTHWEST FOUNDATION FOR RESEARCH & EDUCATION	N CM0735607	22,162
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM0735107	13,345
R.R.P.	STANFORD RESEARCH INSTITUTE	N CM1748500	247,800
D.T.P.	STARKS ASSOCIATES, INC.	N CM0735716	19,282
D.T.P.	STARKS ASSOCIATES, INC.	N CM0735719	23,221
D.T.P.	STARKS ASSOCIATES, INC.	N CM8720600	543,205
D.T.P.	STATE UNIVERSITY OF NEW YORK	N CM2757000	215,725
A.P.	TEXAS A&M RESEARCH FOUNDATION	N CM3753600	2,225
D.T.P.	VSE, CORPORATION	N CM0725100	133,393

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	WARNER LAMBERT	N CM3761400	542,434
	TOTAL		6,205,600

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	ALABAMA, UNIVERSITY OF	N CP9561600B	8,250
D.T.P.	ARIZONA, UNIVERSITY OF	N CM1749700	328,419
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	220,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	371,250
D.T.P.	BIOTECH RESEARCH LABORATORIES, INC.	N CM3755800	121,371
D.T.P.	BRISTOL LABORATORIES	N CM3755600	85,564
D.T.P.	CALIFORNIA, UNIVERSITY OF	N CM0742000	54,900
D.T.P.	CANCER THERAPY & RESEARCH FND. OF SOUTH TEXAS	N CM0732700	66,600
A.P.	CHARLES RIVER BREEDING LABS.	N CM1749800	27,126
A.P.	CHARLES RIVER BREEDING LABS.	N CM3752600	49,146
A.P.	CHARLES RIVER BREEDING LABS.	N CM5059800	21,989
A.P.	CHARLES RIVER BREEDING LABS.	N CM8721200	44,000
A.P.	CHARLES RIVER BREEDING LABS.	N CM9016300	27,401
A.P.	CHARLES RIVER BREEDING LABS.	N CM9722900	210,265
D.T.P.	ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900	323,350
A.P.	HARLAN INDUSTRIES	N CM5059100	13,101
A.P.	HARLAN SPRAGUE DAWLEY, INC.	N CM2391100	380,681
BRMP	HARLAN SPRAGUE DAWLEY, INC.	N CM2391100I	140,000
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	496,995
D.T.P.	INSTITUT JULES BORDET	N CM0735000	159,429
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	6,400
A.P.	KING ANIMAL LABORATORY	N CM1749900	16,082
A.P.	LABORATORY SUPPLY COMPANY, INC.	N CM5057700	17,567
A.P.	MASON RESEARCH INSTITUTE	N CM8716400	7,984

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	426,926
A.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700B	30,500
D.T.P.	MAYO FOUNDATION	N CM0741900	36,000
D.T.P.	MICROBIAL CHEMISTRY RESEARCH FDN.	N CM5700900	106,400
A.P.	MICROBIOLOGICAL ASSOCIATES	N CM9724600	175,976
A.P.	MISSOURI, UNIVERSITY OF	N CM2753400	36,427
A.P.	MISSOURI, UNIVERSITY OF	N CM8715700	92,127
A.P.	MURPHY BREEDING LABS., INC.	N CM5057900A	17,978
A.P.	NORTHWESTERN UNIVERSITY	N CM1736300	16,476
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000	83,590
A.P.	PROGRAM RESOURCES, INC.	N CO2391000P	60,000
A.P.	SIMONSEN LABORATORIES	N CM5057800	21,930
A.P.	SIMONSEN LABORATORIES	N CM9724700	237,625
A.P.	SOUTHERN ANIMAL FARMS	N CM5059900	17,017
A.P.	SOUTHERN ANIMAL FARMS	N CM9724500	100,557
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM3755200	139,584
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	526,652
R.R.P.	STANFORD RESEARCH INSTITUTE	N CM1748500	165,200
A.P.	TEXAS A&M RESEARCH FOUNDATION	N CM3753600	24,478
D.T.P.	VSE, CORPORATION	N CM0725100	552,626
D.T.P.	WARNER LAMBERT	N CM3761400	95,724
	TOTAL		6,161,663

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT		#	DOLLAR LEVEL
	STAGE II FORMULATION:			
D.T.P.	BEN VENUE LABORATORIES, INC.	N	CM2750800	110,998
D.T.P.	ROXANNE LABORATORIES, INC.	N	CM6705300	15,800
C.O.P.	VETERANS ADMINISTRATION MEDICAL CENTER	Y	CM3025600	5,400
D.T.P.	YAMANOUCHI PHARMACEUTICAL CO.	N	CM9730700	48,620
	TOTAL			180,818

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#		DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:			
A.P.	ALABAMA, UNIVERSITY OF	N	CP9561600B	3,750
D.T.P.	ARIZONA, UNIVERSITY OF	N	CM1749700	36,491
D.T.P.	ARTHUR D. LITTLE, INC.	N	CM0734600	100,000
D.T.P.	ARTHUR D. LITTLE, INC.	N	CM1739700	118,720
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N	CM0726600	168,750
D.T.P.	BIOTECH RESEARCH LABORATORIES, INC.	N	CM3755800	28,009
D.T.P.	CALIFORNIA, UNIVERSITY OF	N	CM0742000	6,100
D.T.P.	CANCER THERAPY & RESEARCH FND. OF SOUTH TEXAS	N	CM0732700	7,400
A.P.	CHARLES RIVER BREEDING LABS.	N	CM1749800	12,330
A.P.	CHARLES RIVER BREEDING LABS.	N	CM3752600	49,146
A.P.	CHARLES RIVER BREEDING LABS.	N	CM5059800	9,995
A.P.	CHARLES RIVER BREEDING LABS.	N	CM8721200	20,000
A.P.	CHARLES RIVER BREEDING LABS.	N	CM9016300	12,455
A.P.	CHARLES RIVER BREEDING LABS.	N	CM9722900	95,575
D.T.P.	ENVIRONMENTAL PROTECTION AGENCY	Y	CM2010900	99,492
A.P.	HARLAN INDUSTRIES	N	CM5059100	5,955
D.T.P.	IIT RESEARCH INSTITUTE	N	CM9731600	76,999
D.T.P.	INSTITUT JULES BORDET	N	CM0735000	39,857
D.T.P.	INSTITUTE OF CANCER RESEARCH	N	CM4373600	100,000
A.P.	KING ANIMAL LABORATORY	N	CM1749900	7,310
A.P.	LABORATORY SUPPLY COMPANY, INC.	N	CM5057700	7,985
BRMP	LITTON BIONETICS, INC.	N	CM1580800	98,500
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N	CM0732500	15,000
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N	CM9731700	167,971

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700B	12,000
D.T.P.	MAYO FOUNDATION	N CM0741900	4,000
BRMP	MELOY LABORATORIES, INC.	N CM1575700	55,500
A.P.	MICROBIOLOGICAL ASSOCIATES	N CM9724600	79,989
A.P.	MISSOURI, UNIVERSITY OF	N CM2753400	16,558
A.P.	MISSOURI, UNIVERSITY OF	N CM8715700	41,876
A.P.	MURPHY BREEDING LABS., INC.	N CM5057900A	8,172
A.P.	NORTHWESTERN UNIVERSITY	N CM1736300	7,489
A.P.	PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000	37,995
A.P.	SIMONSEN LABORATORIES	N CM5057800	9,968
A.P.	SIMONSEN LABORATORIES	N CM9724700	108,011
A.P.	SOUTHERN ANIMAL FARMS	N CM5059900	7,735
A.P.	SOUTHERN ANIMAL FARMS	N CM9724500	45,708
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	427,904
A.P.	TEXAS A&M RESEARCH FOUNDATION	N CM3753600	15,577
D.T.P.	VSE, CORPORATION	N CM0725100	190,561
	TOTAL		2,356,833

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	AEROJET STRATEGIC PROPULSION CO.	N CM1749000	197,872
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N CM1749200	152,550
D.T.P.	ASH STEVENS, INC.	N CM1748800	218,724
D.T.P.	FLOW LABORATORIES, INC.	N CM2750500	135,196
BRMP	HYBRITECH, INC.	N CM2601000	210,000
ISDT	LITTON BIONETICS, INC.	N CM0572400	400,000
ISDT	LITTON BIONETICS, INC.	N CM0734700	36,223
D.T.P.	MIDWEST RESEARCH INSTITUTE	N CM8723400	14,429
D.T.P.	MONSANTO RESEARCH CORPORATION	N CM2751600	61,508
D.T.P.	PHARM-ECO	N CM1748700	136,110
D.T.P.	PROGRAM RESOURCES, INC.	N C02391000C	200,000
A.P.	PROGRAM RESOURCES, INC.	N C02391000P	60,000
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM2751500	197,318
BRMP	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N CM2560000	175,000
D.T.P.	SRI INTERNATIONAL	N CM2756000	245,999
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM8718300	19,483
D.T.P.	STARKS ASSOCIATES, INC.	N CM1737400	272,957
D.T.P.	WARNER LAMBERT	N CM1749100	164,806
	TOTAL		2,898,175

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PHARMACOLOGY/TOXICOLOGY:		
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM1736500	980,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM3753500	1,325,124
A.P.	MASON RESEARCH INSTITUTE	N CM8716400	76,846
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N CM8716100	30,468
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	32,916
A.P.	TEXAS A&M RESEARCH FOUNDATION	N CM3753600	2,225
	TOTAL		2,447,579

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	AEROJET STRATEGIC PROPULSION CO.	N CM1749000	197,872
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N CM1749200	152,550
D.T.P.	ASH STEVENS, INC.	N CM1748800	218,724
D.T.P.	BEN VENUE LABORATORIES, INC.	N CM2750800	998,978
D.T.P.	FLOW LABORATORIES, INC.	N CM1739800	346,099
D.T.P.	FLOW LABORATORIES, INC.	N CM2750500	45,065
D.T.P.	GEORGIA, UNIVERSITY OF	N CM2740100	128,704
BRMP	HYBRITECH, INC.	N CM2601000	90,000
C.D.P.	LITTON BIONETICS, INC.	N CM3757100	21,965
D.T.P.	MIDWEST RESEARCH INSTITUTE	N CM8723400	57,715
D.T.P.	MONSANTO RESEARCH CORPORATION	N CM2751600	553,570
D.T.P.	PHARM-ECO	N CM1748700	136,110
D.T.P.	PROGRAM RESOURCES, INC.	N CO2391000C	300,000
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N CM2751500	49,329
D.T.P.	ROXANNE LABORATORIES, INC.	N CM6705300	63,200
BRMP	SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH	N CM2560000	75,000
D.T.P.	STANFORD RESEARCH INSTITUTE	N CM8718300	77,934
D.T.P.	STARKS ASSOCIATES, INC.	N CM1737400	272,957
D.T.P.	WARNER LAMBERT	N CM1749100	164,806
D.T.P.	YAMANOUCHI PHARMACEUTICAL CO.	N CM9730700	194,480
	TOTAL		4,145,058

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#		DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES:			
R.R.P.	BUREAU OF RADIOLOGICAL HEALTH, FDA	Y	CM2011500	450,000
R.R.P.	ENERGY, DEPARTMENT OF-OAK RIDGE NATIONAL LABS	Y	CM2011100	177,130
R.R.P.	ENERGY, DEPARTMENT OF-OAK RIDGE NATIONAL LABS	Y	CM2011200	195,619
R.R.P.	ENERGY, DEPARTMENT OF-OAK RIDGE NATIONAL LABS	Y	CM2011300	196,160
R.R.P.	ENERGY, DEPARTMENT OF	Y	CO0032000B	452,500
R.R.P.	ENERGY, DEPARTMENT OF-BROOKHAVEN NAT'L LABS.	Y	CO1071100B	238,000
R.R.P.	ENERGY, DEPARTMENT OF-BROOKHAVEN NAT'L LABS.	Y	CO1071200B	214,000
ISDT	HEM RESEARCH, INC.	N	CM2560800	368,164
ISDT	LITTON BIONETICS, INC.	N	CM0734700	36,223
C.O.P.	LITTON BIONETICS, INC.	N	CM1573700	84,000
ISDT	LITTON BIONETICS, INC.	N	CM2561600	579,507
C.O.P.	LITTON BIONETICS, INC.	N	CM3757100	197,689
A.P.	MASON RESEARCH INSTITUTE	N	CM8716400	14,970
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y	CM2020000	176,000
C.O.P.	NAVY, DEPARTMENT OF	Y	CM1020100	320,000
ISDT	SMALL BUSINESS ADMINISTRATION	N	CM3760900	73,229
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N	CM9730900	16,458
R.R.P.	UTAH, UNIVERSITY OF	N	CO2391700	770,000
C.O.P.	VETERANS ADMINISTRATION MEDICAL CENTER	Y	CM3025600	21,600
	TOTAL			4,581,249

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:		
D.T.P.	ARTHUR D. LITTLE, INC.	N CM0734600	20,000
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N CM0726600	33,750
R.R.P.	ENERGY, DEPARTMENT OF-LAWRENCE BERKELEY LAB	Y CM2011000	66,402
D.T.P.	IIT RESEARCH INSTITUTE	N CM9731600	21,000
C.O.P.	LITTON BIONETICS, INC.	N CM1573700	36,000
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700	69,988
A.P.	MASON RESEARCH INSTITUTE/EG&G	N CM9731700B	5,000
R.R.P.	MASSACHUSETTS GENERAL HOSPITAL	N CM2753200	84,198
C.O.P.	ORKAND CORPORATION	N CM3601000	266,379
R.R.P.	PENNSYLVANIA, UNIVERSITY OF	N CM2752900	26,481
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM3755200	114,205
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N CM9730900	625,399
R.R.P.	TEXAS, UNIVERSITY OF, M.D. ANDERSON	N CM2753100	77,478
D.T.P.	VSE, CORPORATION	N CM0725100	76,224
	TOTAL		1,522,504

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT		#	DOLLAR LEVEL
	PROGRAM MANAGEMENT:			
CTEP	EMMES CORPORATION	N	CM1737100	86,248
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N	CM1734900	2,500
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N	CM2205400	3,200
O.D.	JWK INTERNATIONAL CORP.	N	CM2560200	226,896
A.P.	NATIONAL ACADEMY OF SCIENCES	N	CM5385000	30,000
C.D.P.	NATIONAL NAVAL MEDICAL CENTER	Y	CM2020000	44,000
C.D.P.	NAVY, DEPARTMENT OF	Y	CM1020100	80,000
A.P.	PROGRAM RESOURCES, INC.	N	CO2391000P	90,000
CTEP	SOCIAL & SCIENTIFIC SYSTEMS, INC.	N	CM1752100	53,320
CTEP	SOCIAL & SCIENTIFIC SYSTEMS, INC.	N	CM2560600	90,000
	TOTAL			706,164

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
R.R.P.	ARIZONA, UNIVERSITY OF	N CM1752200	71,125
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	2,400
CTEP	JOHNS HOPKINS UNIVERSITY	N CM2750900	116,700
CTEP	MARYLAND, UNIVERSITY OF	N CM2754100	111,188
R.R.P.	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	N CM2752500	74,599
CTEP	MAYO FOUNDATION	N CM2754800	153,250
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM2754600	120,662
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N CM2754000	101,329
D.T.P.	SRI INTERNATIONAL	N CM2756000	27,333
R.R.P.	STANFORD UNIVERSITY	N CM1748000	68,827
R.R.P.	TEXAS, UNIVERSITY OF	N CM1752400	78,911
CTEP	TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER	N CM2754200	122,019
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM2755000	168,253
R.R.P.	UTAH, UNIVERSITY OF	N CM1752300	93,455
CTEP	VERMONT, UNIVERSITY OF	N CM2754700	145,593
CTEP	WAYNE STATE UNIVERSITY	N CM2755100	127,644
CTEP	WISCONSIN, UNIVERSITY OF	N CM2754900	150,335
	TOTAL		1,733,623

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:		
R.R.P.	HOWARD UNIVERSITY	N CM2754300	89,060
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400	2,400
R.R.P.	MASSACHUSETTS GENERAL HOSPITAL	N CM1748100	44,812
R.R.P.	MAYO FOUNDATION	N CM2752800	98,465
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM0733700	151,600
CTEP	MICHIGAN, UNIVERSITY OF	N CM0740500	142,373
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y CM2020000	37,400
R.R.P.	SOUTHERN CALIFORNIA, UNIVERSITY OF	N CM2748300	64,256
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM0740600	174,166
CTEP	WAYNE STATE UNIVERSITY	N CM0740400	138,793
	TOTAL		943,325

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N CM0733700	124,037
CTEP	MICHIGAN, UNIVERSITY OF	N CM0740500	116,487
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y CM2020000	182,600
C.O.P.	NAVY, DEPARTMENT OF	Y CM1020000	213,000
C.O.P.	NAVY, DEPARTMENT OF	Y CM1020100	400,000
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N CM0740600	142,499
CTEP	WAYNE STATE UNIVERSITY	N CM0740400	113,558
	TOTAL		1,292,181

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS - OTHER RESEARCH:		
R.R.P.	CALIFORNIA, UNIVERSITY OF	N CM9731500	940,377
R.R.P.	FOX CHASE CANCER CENTER	N CM9731400	1,016,197
R.R.P.	HEALTH RESEARCH, INC.	N CM9731100	67,876
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N CM1734900	22,500
CTEP	MOUNT SINAI MEDICAL CENTER	N AI2266900	53,000
R.R.P.	WASHINGTON, UNIVERSITY OF	N CM9728200	871,950
	TOTAL		2,971,900

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1983
AS OF 05/31/83

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
R.R.P.	ALLEGHENY-SINGER RESEARCH CORPORATION	N CM3751200	289,655
R.R.P.	ARIZONA, UNIVERSITY OF	N CM1752200	71,125
CTEP	ARMED FORCES INSTITUTE OF PATHOLOGY	Y CM3011800	12,000
R.R.P.	BUREAU OF RADIOLOGICAL HEALTH, FDA	Y CM2010700	126,780
CTEP	EMMES CORPORATION	N CM1737100	258,744
CTEP	EMMES CORPORATION	N CM8719300	348,102
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N CM2751000	218,441
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N CP0102500B	110,000
R.R.P.	MASSACHUSETTS INSTITUTE OF TECHNOLOGY	N CM2752500	74,599
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N CM8716100	91,404
R.R.P.	STANFORD UNIVERSITY	N CM1748000	68,827
R.R.P.	TEXAS, UNIVERSITY OF	N CM1752400	78,911
R.R.P.	UTAH, UNIVERSITY OF	N CM1752300	93,455
	TOTAL		1,842,043

TABLE III

ANALYSIS OF ACTIVITIES BY CONTRACTS
FOR FISCAL YEAR 1983

AS OF 05/31/83

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
AEROJET STRATEGIC PROPULSION CO.	N CM1749000 D.T.P.		395,743
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	197,872
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	197,872
ALABAMA, UNIVERSITY OF	N CP9561600B A.P.		15,000
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	3,000
BASIC SCREEN ANIMALS		* 55.00*	8,250
VERIFICATION SCREEN ANIMALS		* 25.00*	3,750
ALABAMA, UNIVERSITY OF	N CM0735518 D.T.P.		9,437
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	9,437
ALABAMA, UNIVERSITY OF	N CM0735517 D.T.P.		10,657
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	10,657
ALABAMA, UNIVERSITY OF	N CM0735516 D.T.P.		13,325
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	13,325
ALABAMA, UNIVERSITY OF	N CM0735515 D.T.P.		14,802
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	14,802
ALABAMA, UNIVERSITY OF	N CM2757100 D.T.P.		138,502
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	138,502

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ALDRICH CHEMICAL COMPANY, INC.	N CM1749200 D.T.P.		305,100
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	152,550
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	152,550
ALLEGHENY-SINGER RESEARCH CORPORATION	N CM3751200 R.R.P.		289,655
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	289,655
NON-SPEC SOL. TUM. HYPERTHERMIA		95.00	275,172
NON-SPEC SOL. TUM. DATA PROC. & SUPP.		5.00	14,483
ARIZONA, UNIVERSITY OF	N CM1749700 D.T.P.		364,910
BASIC SCREEN PRIMARY SCREENING, IN VITRO		* 90.00*	328,419
VERIFICATION SCREEN DETAILED DRUG EVALUATION, IN VITRO		* 10.00*	36,491
ARIZONA, UNIVERSITY OF	N CM1752200 R.R.P.		142,249
PHASE I CLINICAL TRIALS NON-SPECIFIC RAD.		* 50.00*	71,125
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC HYPERTHERMIA		* 50.00*	71,125
ARMED FORCES INSTITUTE OF	Y CM3011800 CTEP		12,000
CLINICAL TRIALS SUPPORTIVE RESEARCH TESTICULAR PATHOLOGY SUPPORT		*100.00*	12,000
ARTHUR D. LITTLE, INC.	N CM0734600 D.T.P.		400,000
ACQUISITION OF MATERIALS BIOASSAY OF NATURAL PRODUCTS		* 15.00*	60,000
BASIC SCREEN		* 55.00*	220,000
PRIMARY SCREENING, IN VIVO		50.00	200,000
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	20,000
VERIFICATION SCREEN DETAILED DRUG EVALUATION, IN VIVO		* 25.00*	100,000
TREATMENT STUDIES CHEMOTHERAPY		* 5.00*	20,000
ARTHUR D. LITTLE, INC.	N CM1739700 D.T.P.		118,720
VERIFICATION SCREEN BIOCHEM TESTS		*100.00*	118,720

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ASH STEVENS, INC.	N CM1748800 D.T.P.		437,448
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	218,724
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	218,724
PRODUCTION, SYNTHETICS			
BATTELLE MEMORIAL INSTITUTE	N CM0726600 D.T.P.		675,000
ACQUISITION OF MATERIALS		* 15.00*	101,250
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 55.00*	371,250
PRIMARY SCREENING, IN VIVO		50.00	337,500
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	33,750
VERIFICATION SCREEN		* 25.00*	168,750
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 5.00*	33,750
CHEMOTHERAPY			
BATTELLE MEMORIAL INSTITUTE	N CM1736500 D.T.P.		980,000
PHARMACOLOGY/TOXICOLOGY		*100.00*	980,000
PROTOCOL TOXICITY STUDIES		50.00	490,000
SPECIAL TOXICITY STUDIES		34.00	333,200
ANIMALS		1.00	9,800
DATA PROCESSING AND SUPPORT		15.00	147,000
BATTELLE MEMORIAL INSTITUTE	N CM3753500 D.T.P.		1,325,124
PHARMACOLOGY/TOXICOLOGY		*100.00*	1,325,124
PROTOCOL TOXICITY STUDIES		50.00	662,562
SPECIAL TOXICITY STUDIES		34.00	450,542
ANIMALS		1.00	13,251
DATA PROCESSING AND SUPPORT		15.00	198,769
BEN VENUE LABORATORIES, INC.	N CM2750800 D.T.P.		1,109,975
FORMULATION		* 10.00*	110,998
DEVEL. OF EXP. FORMULATIONS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	998,978
FORMULATION		75.00	832,481
ANALYTICAL AND QUALITY CONTROL		15.00	166,496
BIOTECH RESEARCH LABORATORIES, INC.	N CM3755800 D.T.P..		186,724
ACQUISITION OF MATERIALS		* 20.00*	37,345
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 65.00*	121,371
PRIMARY SCREENING, IN VITRO		5.00	9,336
PRIMARY SCREENING, IN VIVO		55.00	102,698

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
DATA PROCESSING AND SUPPORT		5.00	9,336
VERIFICATION SCREEN		* 15.00*	28,009
DETAILED DRUG EVALUATION, IN VIVO			
BRISTOL LABORATORIES	N CM3755600 D.T.P.		570,425
ACQUISITION OF MATERIALS		* 85.00*	484,861
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	473,453
DATA PROCESSING AND SUPPORT		2.00	11,409
BASIC SCREEN		* 15.00*	85,564
PRIMARY SCREENING, IN VITRO		10.00	57,043
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	28,521
BUREAU OF RADIOLOGICAL HEALTH,	Y CM2010700 R.R.P.		126,780
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	126,780
NON-SPEC SOL. TUM. HYPERTHERMIA			
BUREAU OF RADIOLOGICAL HEALTH,	Y CM2011500 R.R.P.		450,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	450,000
RADIOBIOLOGY		100.00	450,000
CALIFORNIA, UNIVERSITY OF	N CM0742000 D.T.P.		61,000
BASIC SCREEN		* 90.00*	54,900
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	6,100
DETAILED DRUG EVALUATION, IN VITRO			
CALIFORNIA, UNIVERSITY OF	N CM9731500 R.R.P.		940,377
OTHER CLINICAL TRIALS RESEARCH		*100.00*	940,377
NON-SPECIFIC HARDWARE DEVEL.			
CANCER THERAPY & RESEARCH FND. OF	N CM0732700 D.T.P.		74,000
BASIC SCREEN		* 90.00*	66,600
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	7,400
DETAILED DRUG EVALUATION, IN VITRO			
CHARLES RIVER BREEDING LABS.	N CM1749800 A.P.		49,320
ACQUISITION OF MATERIALS		* 20.00*	9,864
ANIMALS			
BASIC SCREEN		* 55.00*	27,126
ANIMALS			
VERIFICATION SCREEN		* 25.00*	12,330
ANIMALS			

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
CHARLES RIVER BREEDING LABS.	N CM9016300 A.P.			49,820
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	9,964	
BASIC SCREEN ANIMALS		* 55.00*	27,401	
VERIFICATION SCREEN ANIMALS		* 25.00*	12,455	
CHARLES RIVER BREEDING LABS.	N CM3752600 A.P.			98,292
BASIC SCREEN ANIMALS		* 50.00*	49,146	
VERIFICATION SCREEN ANIMALS		* 50.00*	49,146	
CHARLES RIVER BREEDING LABS.	N CM5059800 A.P.			39,980
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	7,996	
BASIC SCREEN ANIMALS		* 55.00*	21,989	
VERIFICATION SCREEN ANIMALS		* 25.00*	9,995	
CHARLES RIVER BREEDING LABS.	N CM8721200 A.P.			80,000
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	16,000	
BASIC SCREEN ANIMALS		* 55.00*	44,000	
VERIFICATION SCREEN ANIMALS		* 25.00*	20,000	
CHARLES RIVER BREEDING LABS.	N CM9722900 A.P.			382,300
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	76,460	
BASIC SCREEN ANIMALS		* 55.00*	210,265	
VERIFICATION SCREEN ANIMALS		* 25.00*	95,575	
CHEMICAL ABSTRACTS SERVICE	N CM4372200 D.T.P.			323,000
ACQUISITION OF MATERIALS DATA PROCESSING AND SUPPORT		*100.00*	323,000	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
EMMES CORPORATION	N CM1737100 CTEP		344,992
PROGRAM MANAGEMENT		* 25.00*	86,248
ADMINISTRATION		15.00	51,749
COMMUNICATION AND EDUCATION		10.00	34,499
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 75.00*	258,744
COLORECTAL	DATA PROC. & SUPP.	10.00	34,499
HEAD AND NECK	DATA PROC. & SUPP.	18.00	62,099
LUNG	DATA PROC. & SUPP.	25.00	86,248
PANCREATIC	DATA PROC. & SUPP.	2.00	6,900
STOMACH	DATA PROC. & SUPP.	10.00	34,499
TESTICULAR	DATA PROC. & SUPP.	10.00	34,499
EMMES CORPORATION	N CM8719300 CTEP		348,102
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	348,102
LUNG	DATA PROC. & SUPP.		
ENERGY, DEPARTMENT OF	Y C00032000B R.R.P.		452,500
CELLULAR/SUBCELLULAR STUDIES		*100.00*	452,500
RADIOBIOLOGY		100.00	452,500
ENERGY, DEPARTMENT OF-BROOKHAVEN NAT'L	Y C01071100B R.R.P.		238,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	238,000
RADIOBIOLOGY		100.00	238,000
ENERGY, DEPARTMENT OF-BROOKHAVEN NAT'L	Y C01071200B R.R.P.		214,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	214,000
RADIOBIOLOGY		100.00	214,000
ENERGY, DEPARTMENT OF-LAWRENCE BERKELEY	Y CM2011000 R.R.P.		66,402
TREATMENT STUDIES		*100.00*	66,402
RADIATION PHYSICS			
ENERGY, DEPARTMENT OF-OAK RIDGE	Y CM2011200 R.R.P.		195,619
CELLULAR/SUBCELLULAR STUDIES		*100.00*	195,619
RADIOBIOLOGY		100.00	195,619
ENERGY, DEPARTMENT OF-OAK RIDGE	Y CM2011100 R.R.P.		177,130
CELLULAR/SUBCELLULAR STUDIES		*100.00*	177,130
RADIOBIOLOGY		100.00	177,130

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ENERGY, DEPARTMENT OF-OAK RIDGE	Y CM2011300 R.R.P.		196,160
CELLULAR/SUBCELLULAR STUDIES		*100.00*	196,160
RADIOBIOLOGY		100.00	196,160
ENVIRONMENTAL PROTECTION AGENCY	Y CM2010900 D.T.P.		497,462
ACQUISITION OF MATERIALS		* 15.00*	74,619
DATA PROCESSING AND SUPPORT			
BASIC SCREEN		* 65.00*	323,350
DATA PROCESSING AND SUPPORT			
VERIFICATION SCREEN		* 20.00*	99,492
DATA PROCESSING AND SUPPORT			
FLOW LABORATORIES, INC.	N CM1739800 D.T.P.		346,099
PROD. AND FORM. FOR CLINICAL TRIALS		*100.00*	346,099
DATA PROCESSING AND SUPPORT		30.00	103,830
ST & DIST CLIN DRUGS		70.00	242,269
FLOW LABORATORIES, INC.	N CM2750500 D.T.P.		450,652
ACQUISITION OF MATERIALS		* 60.00*	270,391
DATA PROCESSING AND SUPPORT		10.00	45,065
STORAGE DISTRIBUTION		50.00	225,326
PROCUREMENT OF PRECLINICAL MATERIAL		* 30.00*	135,196
STORAGE DISTRIBUTION			
PROD. AND FORM. FOR CLINICAL TRIALS		* 10.00*	45,065
DATA PROCESSING AND SUPPORT		1.00	4,507
STORAGE DISTRIBUTION		9.00	40,559
FOX CHASE CANCER CENTER	N CM9731400 R.R.P.		1,016,197
OTHER CLINICAL TRIALS RESEARCH		*100.00*	1,016,197
NON-SPECIFIC HARDWARE DEVEL.			
GEORGIA INSTITUTE OF TECHNOLOGY	N CM2751700 D.T.P.		145,028
ACQUISITION OF MATERIALS		*100.00*	145,028
NEW AGENT PROCUREMENT, SYNTHETICS			
GEORGIA, UNIVERSITY OF	N CM2740100 D.T.P.		128,704
PROD. AND FORM. FOR CLINICAL TRIALS		*100.00*	128,704
ANALYTICAL AND QUALITY CONTROL			
HARLAN INDUSTRIES	N CM5059100 A.P.		23,820
ACQUISITION OF MATERIALS		* 20.00*	4,764
ANIMALS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
BASIC SCREEN ANIMALS		* 55.00*	13,101
VERIFICATION SCREEN ANIMALS		* 25.00*	5,955
HARLAN SPRAGUE DAWLEY, INC.	N CM2391100 A.P.		761,362
ACQUISITION OF MATERIALS ANIMALS		* 50.00*	380,681
BASIC SCREEN ANIMALS		* 50.00*	380,681
HARLAN SPRAGUE DAWLEY, INC.	N CM2391100I BRMP		280,000
ACQUISITION OF MATERIALS ANIMALS		* 50.00*	140,000
BASIC SCREEN ANIMALS		* 50.00*	140,000
HEALTH RESEARCH, INC.	N CM9731100 R.R.P.		67,876
OTHER CLINICAL TRIALS RESEARCH NON-SPECIFIC PHOTORADIATION THE		*100.00*	67,876
HEM RESEARCH, INC.	N CM2560800 ISDT		368,164
CELLULAR/SUBCELLULAR STUDIES		*100.00*	368,164
CELL BIOLOGY		80.00	294,531
MOLECULAR BIOLOGY		20.00	73,633
HOWARD UNIVERSITY	N CM2754300 R.R.P.		89,060
PHASE II CLINICAL TRIALS		*100.00*	89,060
COLORECTAL RAD.		30.00	26,718
PANCREATIC RAD.		30.00	26,718
STOMACH RAD.		30.00	26,718
NON-SPEC SOL. TUM. RAD.		10.00	8,906
HYBRITECH, INC.	N CM2601000 BRMP		300,000
PROCUREMENT OF PRECLINICAL MATERIAL RESPONSE MODIFIERS		* 70.00*	210,000
PROD. AND FORM. FOR CLINICAL TRIALS RESPONSE MODIFIERS		* 30.00*	90,000
IIT RESEARCH INSTITUTE	N CM9731600 D.T.P.		699,993
ACQUISITION OF MATERIALS BIOASSAY OF NATURAL PRODUCTS		* 15.00*	104,999
BASIC SCREEN		* 71.00*	496,995
PRIMARY SCREENING, IN VIVO		64.00	447,996

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		7.00	49,000
VERIFICATION SCREEN		* 11.00*	76,999
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 3.00*	21,000
CHEMOTHERAPY			
ILLINOIS, UNIVERSITY OF	N CM3751300 D.T.P.		105,769
ACQUISITION OF MATERIALS		*100.00*	105,769
LITERATURE SURVEILL.			
INFORMATION MANAGEMENT SERVICES,	N CM1734900 CTEP		25,000
PROGRAM MANAGEMENT		* 10.00*	2,500
ADMINISTRATION			
OTHER CLINICAL TRIALS RESEARCH		* 90.00*	22,500
CNS TUMORS DATA PROC. & SUPP.			
INFORMATION MANAGEMENT SERVICES,	N CM2751000 CTEP		218,441
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	218,441
NON-SPECIFIC DATA PROC. & SUPP.			
INFORMATION MANAGEMENT SERVICES,	N CP0102500B CTEP		110,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	110,000
LUNG DATA PROC. & SUPP.			
INSTITUT JULES BORDET	N CM0735000 D.T.P.		199,286
BASIC SCREEN		* 80.00*	159,429
PRIMARY SCREENING, IN VIVO			
VERIFICATION SCREEN		* 20.00*	39,857
DETAILED DRUG EVALUATION, IN VIVO			
INSTITUTE OF CANCER RESEARCH	N CM4373600 D.T.P.		125,000
ACQUISITION OF MATERIALS		* 20.00*	25,000
NEW AGENT PROCUREMENT, SYNTHETICS			
VERIFICATION SCREEN		* 80.00*	100,000
DETAILED DRUG EVALUATION, IN VIVO		70.00	87,500
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	12,500
JAPANESE FOUNDATION FOR CANCER RESEARCH	N CM2205400 O.D.		16,000
ACQUISITION OF MATERIALS		* 10.00*	1,600
NEW AGENT PROCUREMENT, SYNTHETICS		5.00	800
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	800
BASIC SCREEN		* 40.00*	6,400
PRIMARY SCREENING, IN VITRO		2.00	320
PRIMARY SCREENING, IN VIVO		18.00	2,880

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
DRUG EVALUATION		20.00	3,200
PROGRAM MANAGEMENT		* 20.00*	3,200
COMMUNICATION AND EDUCATION		10.00	1,600
RESOURCE DEVELOPMENT		10.00	1,600
PHASE I CLINICAL TRIALS		* 15.00*	2,400
LEUK.-ACUTE GRAN. CHEM.		5.00	800
LEUK.-ACUTE LYMPH. CHEM.		5.00	800
NON-SPECIFIC CHEM.		5.00	800
PHASE II CLINICAL TRIALS		* 15.00*	2,400
LEUK.-ACUTE GRAN. CHEM.		5.00	800
LEUK.-ACUTE LYMPH. CHEM.		5.00	800
NON-SPECIFIC CHEM.		5.00	800
JOHNS HOPKINS UNIVERSITY	N CM2750900 CTEP		116,700
PHASE I CLINICAL TRIALS		*100.00*	116,700
NON-SPECIFIC CHEM.			
JWK INTERNATIONAL CORP.	N CM2560200 O.D.		226,896
PROGRAM MANAGEMENT		*100.00*	226,896
COMMUNICATION AND EDUCATION			
KING ANIMAL LABORATORY	N CM1749900 A.P.		29,240
ACQUISITION OF MATERIALS		* 20.00*	5,848
ANIMALS			
BASIC SCREEN		* 55.00*	16,082
ANIMALS			
VERIFICATION SCREEN		* 25.00*	7,310
ANIMALS			
LABORATORY SUPPLY COMPANY, INC.	N CM5057700 A.P.		31,940
ACQUISITION OF MATERIALS		* 20.00*	6,388
ANIMALS			
BASIC SCREEN		* 55.00*	17,567
ANIMALS			
VERIFICATION SCREEN		* 25.00*	7,985
ANIMALS			
LITTON BIONETICS, INC.	N CM1580800 BRMP		197,000
ACQUISITION OF MATERIALS		* 50.00*	98,500
COLL,STOR,DISTR -BRM			
VERIFICATION SCREEN		* 50.00*	98,500
RESPONSE MODIFIERS			
LITTON BIONETICS, INC.	N CM1573700 C.O.P.		120,000
CELLULAR/SUBCELLULAR STUDIES		* 70.00*	84,000

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
BIOCHEMISTRY		20.00	24,000
CELL BIOLOGY		20.00	24,000
CELL KINETICS		10.00	12,000
MARKERS		10.00	12,000
CARCINOGENESIS		10.00	12,000
TREATMENT STUDIES		* 30.00*	36,000
COMB. MODAL. THERAPY		30.00	36,000
COMB. MODAL. THERAPY - SURG.		10.00	12,000
COMB. MODAL. THERAPY - CHEM.		10.00	12,000
COMB. MODAL. THERAPY - IMM.		10.00	12,000
LITTON BIONETICS, INC.	N CM3757100 C.O.P.		219,654
PROD. AND FORM. FOR CLINICAL TRIALS		* 10.00*	21,965
DATA PROCESSING AND SUPPORT			
CELLULAR/SUBCELLULAR STUDIES		* 90.00*	197,689
IMMUNOLOGY		90.00	197,689
LITTON BIONETICS, INC.	N CM0572400 ISDT		400,000
PROCUREMENT OF PRECLINICAL MATERIAL		*100.00*	400,000
ANIMAL FACILITIES			
LITTON BIONETICS, INC.	N CM0734700 ISDT		72,446
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	36,223
ANTIBODIES/ANTIGENS			
CELLULAR/SUBCELLULAR STUDIES		* 50.00*	36,223
MOLECULAR BIOLOGY		50.00	36,223
LITTON BIONETICS, INC.	N CM2561600 ISDT		579,507
CELLULAR/SUBCELLULAR STUDIES		*100.00*	579,507
BIOCHEMISTRY		30.00	173,852
CELL BIOLOGY		30.00	173,852
IMMUNOBIOLOGY		10.00	57,951
MOLECULAR BIOLOGY		30.00	173,852
MARYLAND, UNIVERSITY OF	N CM2754100 CTEP		111,188
PHASE I CLINICAL TRIALS		*100.00*	111,188
NON-SPECIFIC CHEM.			
MASON RESEARCH INSTITUTE	N CM8716400 A.P.		99,800
BASIC SCREEN		* 8.00*	7,984
TUMOR BANK			
PHARMACOLOGY/TOXICOLOGY		* 77.00*	76,846
PHARMACOLOGY			
CELLULAR/SUBCELLULAR STUDIES		* 15.00*	14,970
BIOCHEMISTRY		15.00	14,970

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MASON RESEARCH INSTITUTE/EG&G	N CM9731700B A.P.		50,000
ACQUISITION OF MATERIALS		* 5.00*	2,500
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 61.00*	30,500
PRIMARY SCREENING, IN VIVO		50.00	25,000
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		11.00	5,500
VERIFICATION SCREEN		* 24.00*	12,000
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 10.00*	5,000
COMB. MODAL. THERAPY		10.00	5,000
COMB. MODAL. THERAPY - SURG.		8.00	4,000
COMB. MODAL. THERAPY - CHEM.		2.00	1,000
MASON RESEARCH INSTITUTE/EG&G	N CM0732500 D.T.P.		15,000
VERIFICATION SCREEN		*100.00*	15,000
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			
MASON RESEARCH INSTITUTE/EG&G	N CM9731700 D.T.P.		699,879
ACQUISITION OF MATERIALS		* 5.00*	34,994
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 61.00*	426,926
PRIMARY SCREENING, IN VIVO		50.00	349,940
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		11.00	76,987
VERIFICATION SCREEN		* 24.00*	167,971
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 10.00*	69,988
COMB. MODAL. THERAPY		10.00	69,988
COMB. MODAL. THERAPY - SURG.		8.00	55,990
COMB. MODAL. THERAPY - CHEM.		2.00	13,998
MASSACHUSETTS GENERAL HOSPITAL	N CM1748100 R.R.P.		44,812
PHASE II CLINICAL TRIALS		*100.00*	44,812
COLORECTAL RAD.		30.00	13,444
PANCREATIC RAD.		30.00	13,444
STOMACH RAD.		30.00	13,444
NON-SPEC SOL. TUM. RAD.		10.00	4,481
MASSACHUSETTS GENERAL HOSPITAL	N CM2753200 R.R.P.		84,198
TREATMENT STUDIES		*100.00*	84,198
RADIATION PHYSICS			
MASSACHUSETTS INSTITUTE OF	N CM2752500 R.R.P.		149,197
PHASE I CLINICAL TRIALS		* 50.00*	74,599
NON-SPECIFIC RAD.			

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 50.00*	74,599
NON-SPECIFIC	HYPERTHERMIA			
MAYO FOUNDATION		N CM2754800 CTEP		153,250
PHASE I CLINICAL TRIALS			*100.00*	153,250
NON-SPECIFIC	CHEM.			
MAYO FOUNDATION		N CM0741900 D.T.P.		40,000
BASIC SCREEN			* 90.00*	36,000
PRIMARY SCREENING, IN VITRO				
VERIFICATION SCREEN			* 10.00*	4,000
DETAILED DRUG EVALUATION, IN VITRO				
MAYO FOUNDATION		N CM2752800 R.R.P.		98,465
PHASE II CLINICAL TRIALS			*100.00*	98,465
COLORECTAL	RAD.		30.00	29,540
PANCREATIC	RAD.		30.00	29,540
STOMACH	RAD.		30.00	29,540
NON-SPEC SOL. TUM.	RAD.		10.00	9,847
MELOY LABORATORIES, INC.		N CM1575700 BRMP		111,000
ACQUISITION OF MATERIALS			* 50.00*	55,500
COLL,STOR,DISTR -BRM				
VERIFICATION SCREEN			* 50.00*	55,500
RESPONSE MODIFIERS				
MELOY LABORATORIES, INC.		N CM1581301 BRMP		270,000
ACQUISITION OF MATERIALS			*100.00*	270,000
RESPONSE MODIFIERS				
MEMORIAL HOSP. FOR CANCER & ALLIED		N CM0733700 CTEP		275,637
PHASE II CLINICAL TRIALS			* 55.00*	151,600
BREAST	CHEM.		15.00	41,346
COLORECTAL	CHEM.		15.00	41,346
HEAD AND NECK	CHEM.		5.00	13,782
LUNG	CHEM.		5.00	13,782
MELANOMA	CHEM.		10.00	27,564
SARCOMAS (GEN.)	CHEM.		5.00	13,782
PHASE III CLINICAL TRIALS			* 45.00*	124,037
BREAST	CHEM.		10.00	27,564
COLORECTAL	CHEM.		5.00	13,782
HEAD AND NECK	CHEM.		5.00	13,782
LUNG	CHEM.		5.00	13,782
MELANOMA	CHEM.		15.00	41,346
SARCOMAS (GEN.)	CHEM.		5.00	13,782

NAME	& & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MEMORIAL HOSP. FOR CANCER & ALLIED	N CM2754600 CTEP		120,662
PHASE I CLINICAL TRIALS		*100.00*	120,662
NON-SPECIFIC	CHEM.		
MICHIGAN, UNIVERSITY OF	N CM0740500 CTEP		258,860
PHASE II CLINICAL TRIALS		* 55.00*	142,373
BREAST	CHEM.	15.00	38,829
COLORECTAL	CHEM.	15.00	38,829
HEAD AND NECK	CHEM.	5.00	12,943
LUNG	CHEM.	5.00	12,943
MELANOMA	CHEM.	10.00	25,886
SARCOMAS (GEN.)	CHEM.	5.00	12,943
PHASE III CLINICAL TRIALS		* 45.00*	116,487
BREAST	CHEM.	10.00	25,886
COLORECTAL	CHEM.	5.00	12,943
HEAD AND NECK	CHEM.	5.00	12,943
LUNG	CHEM.	5.00	12,943
MELANOMA	CHEM.	15.00	38,829
SARCOMAS (GEN.)	CHEM.	5.00	12,943
MICROBIAL CHEMISTRY RESEARCH FDN.	N CM5700900 D.T.P.		266,000
ACQUISITION OF MATERIALS		* 60.00*	159,600
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			
BASIC SCREEN		* 40.00*	106,400
PRIMARY SCREEN., RELATED NEW MODEL DEVEL			
MICROBIOLOGICAL ASSOCIATES	N CM9724600 A.P.		319,956
ACQUISITION OF MATERIALS		* 20.00*	63,991
ANIMALS			
BASIC SCREEN		* 55.00*	175,976
ANIMALS			
VERIFICATION SCREEN		* 25.00*	79,989
ANIMALS			
MIDWEST RESEARCH INSTITUTE	N CM8723400 D.T.P.		72,144
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	14,429
ANALYTICAL AND QUALITY CONTROL			
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	57,715
ANALYTICAL AND QUALITY CONTROL			
MISSOURI, UNIVERSITY OF	N CM2753400 A.P.		66,230
ACQUISITION OF MATERIALS		* 20.00*	13,246
ANIMALS			
BASIC SCREEN		* 55.00*	36,427

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS			
VERIFICATION SCREEN		* 25.00*	16,558
ANIMALS			
MISSOURI, UNIVERSITY OF	N CM8715700 A.P.		167,503
ACQUISITION OF MATERIALS		* 20.00*	33,501
ANIMALS			
BASIC SCREEN		* 55.00*	92,127
ANIMALS			
VERIFICATION SCREEN		* 25.00*	41,876
ANIMALS			
MONSANTO RESEARCH CORPORATION	N CM2751600 D.T.P.		615,078
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	61,508
PLANT PRODUCTS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	553,570
PRODUCTION, SYNTHETICS		15.00	92,262
PRODUCTION, PLANT PRODUCTS		75.00	461,309
MOUNT SINAI MEDICAL CENTER	N AI2266900 CTEP		53,000
OTHER CLINICAL TRIALS RESEARCH		*100.00*	53,000
LEUK.-ACUTE GRAN. DATA PROC. & SUPP.		25.00	13,250
LEUK.-ACUTE LYMPH. DATA PROC. & SUPP.		25.00	13,250
LEUK.-CHRON. GRAN. DATA PROC. & SUPP.		15.00	7,950
OTHER DATA PROC. & SUPP.		35.00	18,550
MURPHY BREEDING LABS., INC.	N CM5057900A A.P.		32,688
ACQUISITION OF MATERIALS		* 20.00*	6,538
ANIMALS			
BASIC SCREEN		* 55.00*	17,978
ANIMALS			
VERIFICATION SCREEN		* 25.00*	8,172
ANIMALS			
NATIONAL ACADEMY OF SCIENCES	N CM5385000 A.P.		30,000
PROGRAM MANAGEMENT		*100.00*	30,000
COMMUNICATION AND EDUCATION			
NATIONAL NAVAL MEDICAL CENTER	Y CM2020000 C.O.P.		440,000
CELLULAR/SUBCELLULAR STUDIES		* 40.00*	176,000
BIOCHEMISTRY		5.00	22,000
CELL BIOLOGY		8.50	37,400
CELL KINETICS		2.50	11,000
IMMUNOBIOLOGY		8.00	35,200
MARKERS		2.50	11,000

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MOLECULAR BIOLOGY		8.50	37,400
RADIOBIOLOGY		2.50	11,000
DATA PROCESSING AND SUPPORT		2.50	11,000
PROGRAM MANAGEMENT		* 10.00*	44,000
ADMINISTRATION		5.00	22,000
COMMUNICATION AND EDUCATION		1.50	6,600
RESOURCE DEVELOPMENT		1.50	6,600
DATA PROCESSING AND SUPPORT		2.00	8,800
PHASE II CLINICAL TRIALS		* 8.50*	37,400
LYMPHOMA-MYCO.FUNG CHEM.		0.50	2,200
LYMPHOMA-MYCO.FUNG IMM.		0.50	2,200
MYELOMA CHEM.		2.50	11,000
LUNG CHEM.		5.00	22,000
PHASE III CLINICAL TRIALS		* 41.50*	182,600
LYMPHOMA-HISTIOCYT CHEM.		4.00	17,600
LYMPHOMA-HODGKIN'S CHEM.		1.00	4,400
LYMPHOMA-MYCO.FUNG RAD.		4.50	19,800
LYMPHOMA-MYCO.FUNG CHEM.		4.50	19,800
NON-HODGKIN LYMPH. CHEM.		2.50	11,000
MYELOMA CHEM.		2.50	11,000
LUNG RAD.		5.00	22,000
LUNG CHEM.		10.00	44,000
PROSTATIC CHEM.		2.50	11,000
TESTICULAR CHEM.		5.00	22,000
NAVY, DEPARTMENT OF	Y CM1020000 C.O.P.		213,000
PHASE III CLINICAL TRIALS		*100.00*	213,000
LYMPHOMA-HISTIOCYT CHEM.		5.00	10,650
LYMPHOMA-HODGKIN'S CHEM.		5.00	10,650
LYMPHOMA-MYCO.FUNG RAD.		10.00	21,300
LYMPHOMA-MYCO.FUNG CHEM.		10.00	21,300
NON-HODGKIN LYMPH. CHEM.		5.00	10,650
BREAST RAD.		5.00	10,650
BREAST CHEM.		5.00	10,650
LUNG RAD.		10.00	21,300
LUNG CHEM.		15.00	31,950
PROSTATIC CHEM.		15.00	31,950
TESTICULAR CHEM.		15.00	31,950
NAVY, DEPARTMENT OF	Y CM1020100 C.O.P.		800,000
CELLULAR/SUBCELLULAR STUDIES		* 40.00*	320,000
BIOCHEMISTRY		5.00	40,000
CELL BIOLOGY		8.50	68,000
CELL KINETICS		2.50	20,000
IMMUNOBIOLOGY		8.00	64,000
MARKERS		2.50	20,000
MOLECULAR BIOLOGY		8.50	68,000
RADIOBIOLOGY		2.50	20,000
DATA PROCESSING AND SUPPORT		2.50	20,000

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PROGRAM MANAGEMENT		* 10.00*	80,000
ADMINISTRATION		5.00	40,000
COMMUNICATION AND EDUCATION		1.50	12,000
RESOURCE DEVELOPMENT		1.50	12,000
DATA PROCESSING AND SUPPORT		2.00	16,000
PHASE III CLINICAL TRIALS		* 50.00*	400,000
LYMPHOMA-HISTIOCYT CHEM.		2.50	20,000
LYMPHOMA-HODGKIN'S CHEM.		2.50	20,000
LYMPHOMA-MYCO.FUNG RAD.		5.00	40,000
LYMPHOMA-MYCO.FUNG CHEM.		5.00	40,000
NON-HODGKIN LYMPH. CHEM.		2.50	20,000
POLYCYTHERMIA VERA RAD.		2.50	20,000
POLYCYTHERMIA VERA CHEM.		2.50	20,000
LUNG RAD.		5.00	40,000
LUNG CHEM.		7.50	60,000
PROSTATIC CHEM.		7.50	60,000
TESTICULAR CHEM.		7.50	60,000
NORTHWESTERN UNIVERSITY	N CM1736300 A.P.		29,956
ACQUISITION OF MATERIALS		* 20.00*	5,991
ANIMALS			
BASIC SCREEN		* 55.00*	16,476
ANIMALS			
VERIFICATION SCREEN		* 25.00*	7,489
ANIMALS			
OHIO STATE UNIVERSITY RESEARCH	N CM2754000 CTEP		101,329
PHASE I CLINICAL TRIALS		*100.00*	101,329
NON-SPECIFIC CHEM.			
OHIO STATE UNIVERSITY RESEARCH	N CM8716100 D.T.P.		121,872
PHARMACOLOGY/TOXICOLOGY		* 25.00*	30,468
ANAL. METH.			
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 75.00*	91,404
NON-SPECIFIC SPEC. PHARM./TOX.			
ORKAND CORPORATION	N CM3601000 C.O.P.		266,379
TREATMENT STUDIES		*100.00*	266,379
DATA PROCESSING AND SUPPORT			
PAPANICOLAOU CANCER RESEARCH INSTITUTE	N CM8723000 A.P.		151,981
ACQUISITION OF MATERIALS		* 20.00*	30,396
ANIMALS			
BASIC SCREEN		* 55.00*	83,590
ANIMALS			
VERIFICATION SCREEN		* 25.00*	37,995

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS			
PENNSYLVANIA, UNIVERSITY OF	N CM2752900 R.R.P.		26,481
TREATMENT STUDIES RADIATION PHYSICS		*100.00*	26,481
PHARM-ECO	N CM1748700 D.T.P.		272,220
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	136,110
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	136,110
POLYSCIENCES, INC.	N CM0730000 D.T.P.		45,000
ACQUISITION OF MATERIALS		*100.00*	45,000
NEW AGENT PROCUREMENT, PLANT PRODUCTS		79.00	35,550
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		10.00	4,500
BIOASSAY OF NATURAL PRODUCTS		11.00	4,950
POLYSCIENCES, INC.	N CM3755700 D.T.P.		392,488
ACQUISITION OF MATERIALS		*100.00*	392,488
NEW AGENT PROCUREMENT, PLANT PRODUCTS		79.00	310,066
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		10.00	39,249
BIOASSAY OF NATURAL PRODUCTS		11.00	43,174
PROGRAM RESOURCES, INC.	N CO2391000P A.P.		300,000
ACQUISITION OF MATERIALS RESPONSE MODIFIERS		* 30.00*	90,000
BASIC SCREEN RESPONSE MODIFIERS		* 20.00*	60,000
PROCUREMENT OF PRECLINICAL MATERIAL RESPONSE MODIFIERS		* 20.00*	60,000
PROGRAM MANAGEMENT		* 30.00*	90,000
ADMINISTRATION		20.00	60,000
DATA PROCESSING AND SUPPORT		10.00	30,000
PROGRAM RESOURCES, INC.	N CO2391000C D.T.P.		500,000
PROCUREMENT OF PRECLINICAL MATERIAL FERMENTATION/ANTIBIOTICS		* 40.00*	200,000
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, FERMENT/ANTIBIOT		* 60.00*	300,000
RESEARCH TRIANGLE INSTITUTE	N CM0735213 D.T.P.		16,004
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	16,004

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
RESEARCH TRIANGLE INSTITUTE	N CM0735206 D.T.P.		886
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	886
RESEARCH TRIANGLE INSTITUTE	N CM0735214 D.T.P.		17,914
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	17,914
RESEARCH TRIANGLE INSTITUTE	N CM0735215 D.T.P.		22,051
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	22,051
RESEARCH TRIANGLE INSTITUTE	N CM2751500 D.T.P.		246,647
PROCUREMENT OF PRECLINICAL MATERIAL RADIOLABELED MATERIALS		* 80.00*	197,318
PROD. AND FORM. FOR CLINICAL TRIALS RADIOLABEL		* 20.00*	49,329
ROXANNE LABORATORIES, INC.	N CM6705300 D.T.P.		79,000
FORMULATION DEVEL. OF EXP. FORMULATIONS		* 20.00*	15,800
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	63,200
FORMULATION		70.00	55,300
ANALYTICAL AND QUALITY CONTROL		10.00	7,900
SIMONSEN LABORATORIES	N CM5057800 A.P.		39,872
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	7,974
BASIC SCREEN ANIMALS		* 55.00*	21,930
VERIFICATION SCREEN ANIMALS		* 25.00*	9,968
SIMONSEN LABORATORIES	N CM9724700 A.P.		432,045
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	86,409
BASIC SCREEN ANIMALS		* 55.00*	237,625
VERIFICATION SCREEN ANIMALS		* 25.00*	108,011

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
SISA, INC.	N CM0735408 D.T.P.		17,809
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	17,809
SISA, INC.	N CM0735409 D.T.P.		13,318
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	13,318
SISA, INC.	N CM0735407 D.T.P.		6,547
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	6,547
SISA, INC.	N CM0735406 D.T.P.		2,340
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	2,340
SLOAN-KETTERING INSTITUTE FOR CANCER	N CM2560000 BRMP		250,000
PROCUREMENT OF PRECLINICAL MATERIAL RESPONSE MODIFIERS		* 70.00*	175,000
PROD. AND FORM. FOR CLINICAL TRIALS RESPONSE MODIFIERS		* 30.00*	75,000
SMALL BUSINESS ADMINISTRATION	N CM1740000 D.T.P.		68,250
ACQUISITION OF MATERIALS DATA PROCESSING AND SUPPORT		*100.00*	68,250
SMALL BUSINESS ADMINISTRATION	N CM3760900 ISDT		244,096
ACQUISITION OF MATERIALS ANIMAL CELLS		* 70.00*	170,867
CELLULAR/SUBCELLULAR STUDIES		* 30.00*	73,229
MOLECULAR BIOLOGY		10.00	24,410
RADIOIMMUNE ASSAY		20.00	48,819
SOCIAL & SCIENTIFIC SYSTEMS, INC.	N CM1752100 CTEP		53,320
PROGRAM MANAGEMENT DATA PROCESSING AND SUPPORT		*100.00*	53,320
SOCIAL & SCIENTIFIC SYSTEMS, INC.	N CM2560600 CTEP		90,000
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	90,000

NAME	# & AREA	PERCENT OF EFFORT		DOLLAR LEVEL
SOUTHERN ANIMAL FARMS	N CM5059900 A.P.			30,940
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	6,188	
BASIC SCREEN ANIMALS		* 55.00*	17,017	
VERIFICATION SCREEN ANIMALS		* 25.00*	7,735	
SOUTHERN ANIMAL FARMS	N CM9724500 A.P.			182,830
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	36,566	
BASIC SCREEN ANIMALS		* 55.00*	100,557	
VERIFICATION SCREEN ANIMALS		* 25.00*	45,708	
SOUTHERN CALIFORNIA, UNIVERSITY OF	N CM2748300 R.R.P.			64,256
PHASE II CLINICAL TRIALS LUNG RAD.		*100.00*	64,256	
SOUTHERN RESEARCH INSTITUTE	N CM0726012 D.T.P.			2,314
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	2,314	
SOUTHERN RESEARCH INSTITUTE	N CM0726014 D.T.P.			29,222
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	29,222	
SOUTHERN RESEARCH INSTITUTE	N CM0726015 D.T.P.			19,057
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	19,057	
SOUTHERN RESEARCH INSTITUTE	N CM0726013 D.T.P.			1,751
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	1,751	
SOUTHERN RESEARCH INSTITUTE	N CM3755200 D.T.P.			253,789
BASIC SCREEN IN VIVO ANALOG SCR.		* 55.00*	139,584	
TREATMENT STUDIES		* 45.00*	114,205	
COMB. MODAL. THERAPY		45.00	114,205	
COMB. MODAL. THERAPY - RAD.		20.00	50,758	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COMB. MODAL. THERAPY - CHEM.		25.00	63,447
SOUTHERN RESEARCH INSTITUTE	N CM9730900 D.T.P.		1,645,786
ACQUISITION OF MATERIALS		* 1.00*	16,458
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 32.00*	526,652
PRIMARY SCREENING, IN VITRO		4.00	65,831
PRIMARY SCREENING, IN VIVO		16.00	263,326
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		10.00	164,579
ANALOG SCREENING		2.00	32,916
VERIFICATION SCREEN		* 26.00*	427,904
DETAILED DRUG EVALUATION, IN VIVO		14.00	230,410
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	164,579
ANTIVIRAL ACTIVITY		2.00	32,916
PHARMACOLOGY/TOXICOLOGY		* 2.00*	32,916
SPECIAL TOXICITY STUDIES		1.00	16,458
BIOASSAY-DRUG METAB.		1.00	16,458
CELLULAR/SUBCELLULAR STUDIES		* 1.00*	16,458
CELL KINETICS		1.00	16,458
TREATMENT STUDIES		* 38.00*	625,399
COMB. MODAL. THERAPY		38.00	625,399
COMB. MODAL. THERAPY - SURG.		17.00	279,784
COMB. MODAL. THERAPY - CHEM.		21.00	345,615
SOUTHWEST FOUNDATION FOR RESEARCH &	N CM0735606 D.T.P.		17,088
ACQUISITION OF MATERIALS		*100.00*	17,088
NEW AGENT PROCUREMENT, SYNTHETICS			
SOUTHWEST FOUNDATION FOR RESEARCH &	N CM0735607 D.T.P.		22,162
ACQUISITION OF MATERIALS		*100.00*	22,162
NEW AGENT PROCUREMENT, SYNTHETICS			
SRI INTERNATIONAL	N CM2756000 D.T.P.		273,332
PROCUREMENT OF PRECLINICAL MATERIAL		* 90.00*	245,999
RADIOLABELED MATERIALS			
PHASE I CLINICAL TRIALS		* 10.00*	27,333
NON-SPECIFIC PHARM./TOX.			
STANFORD RESEARCH INSTITUTE	N CM0735107 D.T.P.		13,345
ACQUISITION OF MATERIALS		*100.00*	13,345
NEW AGENT PROCUREMENT, SYNTHETICS			
STANFORD RESEARCH INSTITUTE	N CM8718300 D.T.P.		97,417
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	19,483
ANALYTICAL AND QUALITY CONTROL			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PROD. AND FORM. FOR CLINICAL TRIALS ANALYTICAL AND QUALITY CONTROL		* 80.00*	77,934
STANFORD RESEARCH INSTITUTE	N CM1748500 R.R.P.		413,000
ACQUISITION OF MATERIALS RAD. MODIFIERS		* 60.00*	247,800
BASIC SCREEN		* 40.00*	165,200
PRIMARY SCREENING, IN VITRO		20.00	82,600
PRIMARY SCREENING, IN VIVO		20.00	82,600
STANFORD UNIVERSITY	N CM1748000 R.R.P.		137,654
PHASE I CLINICAL TRIALS NON-SPECIFIC RAD.		* 50.00*	68,827
CLINICAL TRIALS SUPPORTIVE RESEARCH NON-SPECIFIC HYPERTHERMIA		* 50.00*	68,827
STARKS ASSOCIATES, INC.	N CM0735719 D.T.P.		23,221
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	23,221
STARKS ASSOCIATES, INC.	N CM0735716 D.T.P.		19,282
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	19,282
STARKS ASSOCIATES, INC.	N CM1737400 D.T.P.		545,913
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS		* 50.00*	272,957
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	272,957
STARKS ASSOCIATES, INC.	N CM8720600 D.T.P.		543,205
ACQUISITION OF MATERIALS		*100.00*	543,205
NEW AGENT PROCUREMENT, SYNTHETICS		70.00	380,244
DATA PROCESSING AND SUPPORT		30.00	162,962
STATE UNIVERSITY OF NEW YORK	N CM2757000 D.T.P.		215,725
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	215,725
TEXAS A&M RESEARCH FOUNDATION	N CM3753600 A.P.		44,505
ACQUISITION OF MATERIALS ANIMALS		* 5.00*	2,225
BASIC SCREEN		* 55.00*	24,478

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS				
VERIFICATION SCREEN			* 35.00*	15,577
ANIMALS				
PHARMACOLOGY/TOXICOLOGY			* 5.00*	2,225
ANIMALS				
TEXAS, UNIVERSITY OF, HEALTH SCIENCE		N CM2754200 CTEP		122,019
PHASE I CLINICAL TRIALS			*100.00*	122,019
NON-SPECIFIC	CHEM.			
TEXAS, UNIVERSITY OF, M.D. ANDERSON		N CM2753100 R.R.P.		77,478
TREATMENT STUDIES			*100.00*	77,478
RADIATION PHYSICS				
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N CM0740600 CTEP		316,665
PHASE II CLINICAL TRIALS			* 55.00*	174,166
BREAST	CHEM.		15.00	47,500
COLORECTAL	CHEM.		15.00	47,500
HEAD AND NECK	CHEM.		5.00	15,833
LUNG	CHEM.		5.00	15,833
MELANOMA	CHEM.		10.00	31,667
SARCOMAS (GEN.)	CHEM.		5.00	15,833
PHASE III CLINICAL TRIALS			* 45.00*	142,499
BREAST	CHEM.		10.00	31,667
COLORECTAL	CHEM.		5.00	15,833
HEAD AND NECK	CHEM.		5.00	15,833
LUNG	CHEM.		5.00	15,833
MELANOMA	CHEM.		15.00	47,500
SARCOMAS (GEN.)	CHEM.		5.00	15,833
TEXAS, UNIVERSITY OF, SYSTEM CANCER		N CM2755000 CTEP		168,253
PHASE I CLINICAL TRIALS			*100.00*	168,253
NON-SPECIFIC	CHEM.			
TEXAS, UNIVERSITY OF		N CM1752400 R.R.P.		157,822
PHASE I CLINICAL TRIALS			* 50.00*	78,911
NON-SPECIFIC	RAD.			
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 50.00*	78,911
NON-SPECIFIC	HYPERTHERMIA			
UTAH, UNIVERSITY OF		N CM1752300 R.R.P.		186,910
PHASE I CLINICAL TRIALS			* 50.00*	93,455
NON-SPECIFIC	RAD.			
CLINICAL TRIALS SUPPORTIVE RESEARCH			* 50.00*	93,455
NON-SPECIFIC	HYPERTHERMIA			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
UTAH, UNIVERSITY OF	N CO2391700 R.R.P.		770,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	770,000
RADIOBIOLOGY		100.00	770,000
VERMONT, UNIVERSITY OF	N CM2754700 CTEP		145,593
PHASE I CLINICAL TRIALS		*100.00*	145,593
NON-SPECIFIC CHEM.			
VETERANS ADMINISTRATION	Y CM3025600 C.O.P.		27,000
FORMULATION		* 20.00*	5,400
RESPONSE MODIFIERS			
CELLULAR/SUBCELLULAR STUDIES		* 80.00*	21,600
CELL BIOLOGY		50.00	13,500
IMMUNOBIOLOGY		10.00	2,700
MARKERS		10.00	2,700
MOLECULAR BIOLOGY		10.00	2,700
VSE, CORPORATION	N CM0725100 D.T.P.		952,804
ACQUISITION OF MATERIALS		* 14.00*	133,393
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 58.00*	552,626
PRIMARY SCREENING, IN VITRO		5.00	47,640
PRIMARY SCREENING, IN VIVO		53.00	504,986
VERIFICATION SCREEN		* 20.00*	190,561
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 8.00*	76,224
CHEMOTHERAPY			
WARNER LAMBERT	N CM1749100 D.T.P.		329,611
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	164,806
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	164,806
PRODUCTION, SYNTHETICS			
WARNER LAMBERT	N CM3761400 D.T.P.		638,158
ACQUISITION OF MATERIALS		* 85.00*	542,434
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	529,671
DATA PROCESSING AND SUPPORT		2.00	12,763
BASIC SCREEN		* 15.00*	95,724
PRIMARY SCREENING, IN VITRO		10.00	63,816
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	31,908

NAME		# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
WASHINGTON, UNIVERSITY OF		N CM9728200 R.R.P.		871,950
OTHER CLINICAL TRIALS RESEARCH			*100.00*	871,950
NON-SPECIFIC	HARDWARE DEVEL.			
WAYNE STATE UNIVERSITY		N CM0740400 CTEP		252,351
PHASE II CLINICAL TRIALS			* 55.00*	138,793
BREAST	CHEM.		15.00	37,853
COLORECTAL	CHEM.		15.00	37,853
HEAD AND NECK	CHEM.		5.00	12,618
LUNG	CHEM.		5.00	12,618
MELANOMA	CHEM.		10.00	25,235
SARCOMAS (GEN.)	CHEM.		5.00	12,618
PHASE III CLINICAL TRIALS			* 45.00*	113,558
BREAST	CHEM.		10.00	25,235
COLORECTAL	CHEM.		5.00	12,618
HEAD AND NECK	CHEM.		5.00	12,618
LUNG	CHEM.		5.00	12,618
MELANOMA	CHEM.		15.00	37,853
SARCOMAS (GEN.)	CHEM.		5.00	12,618
WAYNE STATE UNIVERSITY		N CM2755100 CTEP		127,644
PHASE I CLINICAL TRIALS			*100.00*	127,644
NON-SPECIFIC	CHEM.			
WISCONSIN, UNIVERSITY OF		N CM2754900 CTEP		150,335
PHASE I CLINICAL TRIALS			*100.00*	150,335
NON-SPECIFIC	CHEM.			
YAMANOUCHI PHARMACEUTICAL CO.		N CM9730700 D.T.P.		243,100
FORMULATION			* 20.00*	48,620
DEVEL. OF EXP. FORMULATIONS				
PROD. AND FORM. FOR CLINICAL TRIALS			* 80.00*	194,480
FORMULATION			70.00	170,170
ANALYTICAL AND QUALITY CONTROL			10.00	24,310

TABLE IV

DESCRIPTION OF CONTRACTS

IN THE

DIVISION OF CANCER TREATMENT

AEROJET STRATEGIC PROPULSION COMPANY (NO1-CM1-7490)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. The major effort (approximately 90%) of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

ALABAMA, UNIVERSITY OF (NO1-CM0-7355)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

ALABAMA, UNIVERSITY OF (NO1-CM2-7571)

This project is one of three contracts whose objectives are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improve bio-availability by increasing aqueous solubility; (b) increase compound stability; (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles.

ALBANY MEDICAL COLLEGE (NO1-CB5-3940)

This contract is evaluating the therapeutic efficacy of intrapleural BCG in patients with lung cancer. Following surgical resection, patients were randomly assigned to receive intrapleural BCG + INH or INH alone. The design was subsequently altered so that Stage I patients received either BCG + INH, followed by additional doses of BCG, and control patients received BCG + INH alone. This contract will be extended at "no cost" during 1983.

ALBANY MEDICAL COLLEGE (NO1-CM5-7032)

This contract was designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. This contract expired on February 28, 1983 and will not be recompeted.

ALDRICH CHEMICAL COMPANY, INC. (NO1-CM1-7492)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

AMERICAN COLLEGE OF RADIOLOGY (NO1-CM8-7219)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 124 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

AMERICAN TYPE CULTURE COLLECTION (NO1-CM0-5725)

This contract supplies the Government with substantial quantities of well-characterized normal and neoplastic mammalian tissue culture cells and receives, processes, and distributes fresh human leukemic cells and tissues. Cell cultures are grown under specified conditions and all harvested cells are required to be metabolically active and delivered to the Government within one hour. Complete records are maintained on all biological materials handled under the contract. This contract is being recompeted.

ARIZONA, UNIVERSITY OF (NO1-CM1-7497)

This is one of four contracts devoted to the application of a human tumor clonogenic assay to drug screening. Previous effort on this contract has resulted in the development of a quality controlled protocol useful for drug screening with a number of tumor types. Recent work has been confined to those which perform best in the assay: breast, colorectal, kidney, lung, and melanoma. A substantial amount of data has been gathered to support the validity and reproducibility of the assay for new drug screening. Initial screening results have been quite encouraging. A group of compounds which had been negative in the in vivo P388 leukemia system currently used as a pre-screen were tested in the clonogenic assay. Of these 10/77 showed significant activity. Several of these had outstanding in vitro activity and represent novel structural types. Current efforts on the contract are devoted to further drug screening and to a limited number of developmental studies aimed at further improving the protocol.

ARIZONA, UNIVERSITY OF (NO1-CM1-7522)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

ARMED FORCES INSTITUTE OF PATHOLOGY (Y01-CM3-0118)

The purpose of this Interagency Agreement is to provide processing and pathologic examinations of all testicular tumor tissue slides and blocks submitted by the Intergroup Stage I and II Testicular Protocol to the Testicular Tumor Study Group of the Extramural Clinical Trials Program.

ARTHUR D. LITTLE, INC. (NO1-CM0-7257)

The capability for evaluating chemical compounds for radiation sensitizing properties is provided by this resource. Various physico-chemical parameters are determined on a large number of compounds representing a wide range of chemical structures. Compounds showing potential radiosensitizing characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiosensitization using mammalian cell cultures. Compounds which appear to be superior to the standard - misonidazole - will be evaluated in vivo, using mice and/or rats. The effect of the compound in modifying the response to radiation will be measured in three different tumor systems (from the DCT panel of mouse tumor screens as stated in the Treatment Linear Array for Radiosensitizers), each measured by a separate endpoint. The endpoints will include: the regrowth delay of tumors, tumor cell survival and the modification of the radiation dose required for curing 50% of the tumors. All testing will be compared with the standard - misonidazole.

ARTHUR D. LITTLE, INC. (NO1-CMO-7257) - (CONTINUED)

The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds.

This contract should provide new radiosensitizers or leads in developing new types (classes) of radiosensitizing compounds. This contract is currently being recompeted.

ARTHUR D. LITTLE, INC. (NO1-CMO-7302)

This contract utilizes a variety of murine leukemia and solid tumor models in carrying out its major objectives: (1) evaluation of the antitumor activity of structural congeners in order to find which, if any, of a series of closely related structures are worthy of potential development; (2) evaluation of purified natural products studies; (3) combination chemotherapy studies; and (4) special, secondary evaluation studies. In these latter studies the contractor, upon NCI request, plans and conducts studies in response to questions that arise at Decision Network or other meetings of drug development committees or during the toxicologic or clinical investigation of new agents. This contract terminated in March 1983, but was recompeted at a reduced level of effort.

ARTHUR D. LITTLE, INC. (NO1-CMO-7346)

The current level of testing in mice of potential anticancer agents under this contract is approximately 13,500 L1210 equivalent tests per year. The contract provides for *in vivo* testing in the P388 leukemia pre-screen, for evaluation of materials in specified tumor panel models, for detailed evaluations requested by members of the NCI staff, and for characterizations and evaluations of tumor models as directed by the NCI Project Officer. All testing is carried out in accordance with the protocols of the NCI Developmental Therapeutics Program. Materials tested in the P388 leukemia pre-screen are new synthetic compounds and fractions of natural products provided by the NCI. Models of the conventional tumor panel now in use under this contract include the B16 melanocarcinoma, colon 38 carcinoma, and L1210 leukemia. Materials tested in the tumor panel models under this contract are selected by the Developmental Therapeutics Program and consist particularly of materials that have demonstrated activity in the P388 pre-screen. Detailed testing usually involves materials of potential clinical interest and includes route and schedule studies, batch comparisons, and formulation comparisons.

ARTHUR D. LITTLE, INC. (NO1-CM1-7397)

This contract is designed to obtain basic information on the cytotoxic and biochemical effects of new antitumor agents being considered for development to clinical trial. Experiments are conducted (1) to establish whether agents with novel chemical structures have biochemical activities similar to those of clinically evaluated drugs; (2) to answer specific biological questions on new antitumor agents that are raised by the Decision Network Committee; and (3) to provide in vitro biological data to committees and coordinating groups in the Division of Cancer Treatment to aid them in their choice of new agents, or analogs of lead drugs for development.

ARTHUR D. LITTLE, INC. (NO1-CM3-7596)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established antitumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

ARTHUR D. LITTLE, INC. (NO1-CM9-7288)

This contract which had provided KB, P388, L1210, and astrocytoma cytotoxicity assays as an aid in development of potential antitumor agents for the natural products program of DCT, was extended from August 1982 through December 1982 at a minimal level of effort as part of the phase-out of the natural products program. During this period, evaluation of the astrocytoma assay was finalized and documented. This contract terminated December 31, 1982.

ASH STEVENS, INC. (NO1-CM1-7488)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. About 60% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

BANNER GELATIN PRODUCTS CORPORATION (NO1-CM1-7402)

The objectives of this contract are to provide facilities and capabilities for the development and production of soft gelatin capsules containing investigational anticancer agents. The contractor is responsible for conformity to FDA Current Good Manufacturing Practices and for completing all required analytical testing on each product prepared. All products are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BATTELLE MEMORIAL INSTITUTE (NO1-CM0-7266)

This contract conducts in vivo screening of new materials in the P388 leukemia pre-screen as well as the secondary testing of materials of interest to the Developmental Therapeutics Program. This latter testing is conducted in a panel of murine tumor and human xenograft tumor systems. Testing is conducted at a level of approximately 22,500 L1210 equivalents per year. Upon request of the Project Officer, special studies for the detailed evaluation of drugs of interest to the Program tumor panel are conducted, as are studies with new tumor panel systems.

BATTELLE MEMORIAL INSTITUTE (NO1-CM1-7365)

This service type Prime Contract with Battelle is for the supervision of subcontractors carrying out the toxicologic evaluation of potential oncolytic agents, biologic response modifiers, radiosensitizers/protectors and other modalities. Through the Prime Contract mechanism, preclinical toxicologic studies of agents under consideration for potential clinical use are handled under a single management-type contract. The work scope under this contract is comprised of four tasks as follows: Task I - Full Protocol Studies; Task II - High Priority Toxicity Studies (i.e., any portion of the Protocol of the Toxicology Branch); Task III Specific Organ Toxicity Testing; and Task IV - Automation of toxicity data handling, anomaly detection, scheduling of studies, quality assurance auditing of data and financial management. This contract is currently being recompeted.

BEN VENUE LABORATORIES, INC. (NO1-CM2-7508)

This resource contract provides for the development and production of parenteral clinical dosage forms of antitumor agents. The contractor has the capacity for preparing production batches of dry filled, liquid filled and lyophilized sterile products. Specifically, the contractor performs the following services: (1) formulation development of parenteral products; (2) production of sterile products; and (3) quality assurance testing of finished products. All products are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CM3-7558)

This contract provides assistance to the Drug Evaluation Branch in maintaining an orderly flow of materials to screening laboratories and evaluating the test results. They also provide assistance to staff in the scheduling of testing and the evaluation of the data for those materials which have been recommended for further evaluation in additional antitumor test systems. The contractor provides for the coordination of data entry to files which provide a tracking system for the status of materials of interest to the Program. This contract was initiated May 1983.

BOWMAN GRAY SCHOOL OF MEDICINE (NO1-CM3-7603)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

BRISTOL LABORATORIES, INC. (NO1-CM3-7556)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various unique microbes isolated in Bristol's Japanese facility, Bristol Banyu; (2) the use of 10 different in vitro pre-screens to evaluate the fermentations; (3) development of an in vitro assay to assist in quickly isolating the active anticancer agents; (4) dereplication of the materials to determine novelty; (5) chemical isolation of the active component; and (6) production of large quantities of new agents to thoroughly evaluate them in DCT screens. This contract has been re-competed and re-awarded to this contractor.

BUREAU OF RADIOLOGICAL HEALTH, FDA (YO1-CM2-0107)

This Interagency Agreement provides technical support to NCI and to the five contractors participating in the collaborative Phase I evaluation of equipment for the hyperthermic treatment of cancer. The Division of Electronic Products, Bureau of Radiologic Health, FDA, has a number of highly recognized experts in electromagnetic radiation and in ultrasound who are available on a consultative basis to assist in the hyperthermia research program.

BUREAU OF RADIOLOGICAL HEALTH, FDA (YO1-CM2-0115)

This Intra-agency Agreement is for partial support of a continuing study of malignancy as a cause of death in beagle dogs given whole body irradiation during development. This is part of a comprehensive study of the long-term effects of prenatal and postnatal gamma radiation exposures conducted at the Collaborative Radiological Health Laboratory at Colorado State University and sponsored by the FDA Bureau of Radiological Health. Groups of dogs have been exposed to whole-body irradiation of three prenatal and three postnatal ages, specifically at 8, 28 or 55 days postcoitus, and at 2, 70 or 365 days postpartum at doses of either 16 or 83 rad. As of the end of December, 1982 there were about 630 dogs remaining alive, all of them at least 10 years old. It is estimated that 50-75% of these will die of some malignancy in future years. Preliminary projection analyses have been made to predict the likelihood of statistically significant results in future years, and more refined analyses are being made now in collaboration with statisticians at NCI and FDA. This partial support is expected to continue for 4 more years.

BUREAU OF RADIOLOGICAL HEALTH, FDA (Y01-C01-0700)

The purpose of this Intra-agency Agreement is to determine whether 131-iodine exposures are associated with subsequent risk of developing benign or malignant thyroid neoplasms. The study population consists of 6,500 patients under age 20 who received diagnostic 131-iodine between 1946-1967 to test thyroid gland function and for thyroid imaging. The control group consists of 13,000 patients (6,500 clinical comparision patients and 6,500 siblings of exposed group). This contract expired January 31, 1983.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7420)

This is one of four contracts devoted to the application of a human tumor clonogenic assay to drug screening. Previous effort on this contract has resulted in the development of a quality controlled protocol useful for drug screening with a number of tumor types. Recent work has been confined to those which perform best in the assay: breast, colorectal, kidney, lung, and melanoma. A substantial amount of data has been gathered to support the validity and reproducibility of the assay for new drug screening. Initial screening results have been quite encouraging. A group of compounds which had been negative in the in vivo P388 leukemia system currently used as a pre-screen were tested in the clonogenic assay. Of these 10/77 showed significant activity. Several of these had outstanding in vitro activity and represent novel structural types. Current efforts on the contract are devoted to further drug screening and to a limited number of developmental studies aimed at further improving the protocol.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7439-1)

A Phase I trial of human lymphoblastoid interferon (Wellferon®) has been completed. The study showed that the maximum tolerated dose was a total of 30 million units given every 12 hours for 7 days. A partial response was seen in 3 patients, one each with renal cell carcinoma, diffuse histiocytic lymphoma, and Hodgkin's disease. Positive responses to therapy did not correlate with dose level. Cumulative dosages seemed to decrease NK and K cell cytotoxic function by day 7 followed by a return to normal by days 8 and 12. Phase II trials of Wellferon® in patients with multiple myeloma (in conjunction with Duke University and Memorial Sloan Kettering) and breast cancer have been initiated and are ongoing. This contract will expire October 31, 1983.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7439-2)

This contract is to conduct a Phase I/II clinical trial of anti-T cell monoclonal antibodies produced at UCLA in patients with T cell malignancies. These antibodies have shown variable reactivity with tumor cells in patients with the T cell malignancies (T cell ALL, Sezary cells, T cell CLL). Patient accrual has not started and is awaiting production and certification of appropriate material. This contract will expire October 31, 1984.

CALIFORNIA, UNIVERSITY OF (NO1-CMO-7444)

This contract is for the conduct of a Phase I/II clinical trial of anti-T cell monoclonal antibody (T101) in patients with T cell malignancies. This study will determine the dose, biological effect and clinical efficacy of this antibody in patients with refractory T cell malignancies. Provisions to determine the pharmacokinetics of administered antibody as well as a determination of antigenic modulation are included. The protocol has recently been activated and patient accrual is underway.

CALIFORNIA, UNIVERSITY OF (NO1-CM9-7315)

This contract provides for a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support clinical neutron therapy research at UCLA. The proposed facility will be constructed on the grounds of the Wadsworth Veterans Administration Medical Center near the UCLA campus. The cyclotron and associated components of the neutron therapy system are being fabricated in Berkeley, California. Construction of the facility began in November 1982, was 25% complete on March 28, 1983 and is scheduled for completion in December 1983. The Cyclotron Corporation, which is manufacturing the cyclotron and neutron therapy system, filed for bankruptcy under Chapter 11 of the Bankruptcy Code. Work is continuing on the cyclotron under agreement with the Debtors Committee. Delivery of the cyclotron is tentatively scheduled for mid 1984.

CALIFORNIA, UNIVERSITY OF (NO1-CM9-7318)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status. This contract is in the final year of follow-up and will expire October 1983.

CANCER THERAPY AND RESEARCH FOUNDATION OF SOUTH TEXAS (NO1-CMO-7327)

This is one of four contracts devoted to the application of a human tumor clonogenic assay to drug screening. Previous effort on this contract has resulted in the development of a quality controlled protocol useful for drug screening with a number of tumor types. Recent work has been confined to those which perform best in the assay: breast, colorectal, kidney, lung, and melanoma. A substantial amount of data has been gathered to support the validity and reproducibility of the assay for new drug screening. Initial screening results have been quite encouraging. A group of compounds which had been negative in the *in vivo* P388 leukemia system currently used as a pre-screen were tested in the clonogenic assay. Of these 10/77 showed significant activity. Several of these had outstanding *in vitro* activity and represent novel structural types. Current efforts on the contract are devoted to further drug screening and to a limited number of developmental studies aimed at further improving the protocol.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM1-7498)

This procurement contract is designed to furnish 156,000 six-week old first-generation hybrid mice for Developmental Therapeutics Program contract studies. This contract terminated April 1983. A new competition is in progress for recompeting this effort.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM3-7526)

This contract provides for the rederivation of approximately 16 mouse and rat strains and one guinea pig strain on an annual basis. Rederived strains will be distributed to genetic centers for expansion and replacement of producing strains. This contract terminated February 1983. This effort was recompeted and a new award was made to Charles River effective March 1983.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM5-0598)

This procurement contract is designed to furnish 156,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for Developmental Therapeutics Program contract studies. The breeding animals originate from the genetic center at this site. This contract terminated April 1983. A new competition is in progress for recompeting this effort.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM7-7141)

This primary genetic center has as its objectives the development of associated foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterectomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artifically contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a barrier room. Offspring from this second stage are issued to large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. This contract terminated June 1983. A new competition is in progress for recompeting this effort.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM8-7212)

This contract provides for the continual monitoring of the 350 plus associated isolators within the DCT animal program to determine the flora status of these foundation isolators and to check for specified isolator contaminants. In addition, this contract will provide the correct organisms for establishing flora in new isolators.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM9-0163)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers. This contract terminated April 1983. A new competition is in progress for recompeting this effort.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM9-7229)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the government from primary genetic centers.

CHEMICAL ABSTRACTS SERVICE (NO1-CM4-3722)

This contractor operates the NCI's Chemical Information System, a large computerized system holding the structures and associated data of over 350,000 chemical compounds tested by the NCI as anticancer agents. The system is used to determine which actual or potential acquisitions are identical or similar to previous accessions, to maintain inventory control, to monitor the movement of each sample from its receipt through shipment to screener, to allow for online and offline querying of the file, to run a model that predicts activities and toxicities and novelty of potential acquisitions, and to coordinate the actions of the several contractors involved in the pre-screen operations.

CINCINNATI, UNIVERSITY OF (NO1-CM8-7222)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 36 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 3, 1984.

DUKE UNIVERSITY (NO1-CM0-7436)

The Phase I clinical study of human lymphoblastoid interferon (Wellferon®) was concluded and a maximal tolerated dose of 15 million units/m^2 three times per week was determined. No significant tumor responses were seen. NK activity was stimulated by interferon, but paradoxically, repeated high doses of interferon resulted in reduction of NK activity. No major changes in lymphocyte subpopulations were noted. Chronic administration of interferon over a 5 week period did suppress erythropoiesis and granulopoiesis to a similar degree. A Phase II study of Wellferon® in colon cancer has also been completed. This study did not show significant activity for Wellferon® in patients with this malignancy. Phase II trials are also underway in patients with multiple myeloma (in conjunction with the University of California, Los Angeles and Memorial Sloan Kettering) and renal cell carcinoma. This contract will expire October 31, 1983.

DUKE UNIVERSITY MEDICAL CENTER (NO1-CM1-7477)

The Neuropathology Department of this institution functions as a Neuropathology Coordinating Center, providing neuropathologic support for the clinical trials conducted by the Brain Tumor Study Group (BTSG). This center has the responsibility for receiving both the surgical and autopsy material and providing the final pathology diagnosis on all patients randomized to the BTSG protocols. In addition, the Center conducts and reports special studies correlating various histologic features of brain tumors with the natural course of the disease, effect of treatment and various diagnostic and follow-up procedures. This contract has been terminated because of budget cuts which initiated the phase-out of the Brain Tumor Study Group.

DYNAMAC CORPORATION (NO1-CM0-7332)

The objectives of this project are to develop and maintain a systematic literature surveillance effort to identify published compounds which warrant acquisition based on their structural characteristics and biological properties. This contract is monitoring a broad base of chemical, biochemical, biological and patent literature to identify compounds for potential acquisition or task order synthesis.

EMMES CORPORATION (NO1-CM1-7371)

This contract provides operations office support for the Gastrointestinal Tumor Study Group, the Head and Neck Contracts Project, the Intergroup Testicular Cancer Studies, and the Lung Cancer Study Group. Functions include coordination of protocol development, editing and preparing final form of protocols, forms design, randomization, quality control of data, editing and preparing meeting agenda which include clinical trials reports, writing and preparing minutes of meetings, preparing correspondence, record-keeping, and files maintenance.

EMMES CORPORATION (NO1-CM8-7193)

The EMMES Corporation provides the statistical support for the Gastrointestinal Tumor Study Group. They assist in design of protocols, perform statistical analyses of studies, and assist investigators in preparing manuscripts presenting the data.

EMORY UNIVERSITY (NO1-CM2-5603)

This study is evaluating the use of direct and indirect calorimetry in cancer patients and in aged matched "normal" volunteers and patients without malignancies. In addition, other parameters of nutritional status are being performed and will be correlated with the results of the calorimetric studies. This contract is being phased out and will not be recompeted.

ENERGY, DEPARTMENT OF (YO1-CM2-0110)

This contract is part of a collaborative effort to improve treatment planning with neutrons and with charged particles (protons, helium ions, pi-mesons and heavy ions). In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, the collaborative working group is conducting a comparison of treatment plans for selected tumors in each anatomic site. Whenever possible, measurements in patients or phantoms will be made to check the accuracy of the treatment planning.

ENERGY, DEPARTMENT OF (YO1-CM2-0111)

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Radiation-induced Myelogenous Leukemia." This project is concerned with the dose-response relationships and the mechanisms of neutron radiation-induced myelogenous leukemia in mice. Neutrons and other high-LET radiations are considered more effective for induction of tumors than low-LET radiations. Since survivors of the atomic bomb at Hiroshima were exposed to both gamma rays and neutrons, attempts have been made to deduce the leukemogenic effects of neutrons from the Japanese data, but the neutron dose estimates for Hiroshima have recently been questioned and may be revised. It is essential to obtain data for neutron radiation-induced leukemia in experimental animals. Efforts during the first year of this project have been directed toward irradiation of animals in the various dose groups according to the protocol. Neutron irradiations for the dose response portion of the study were begun on April 1, 1982 and were completed on April 30, 1983. No significant mortality has occurred to date. Based on previous studies it is not expected that many mice will develop myelogenous leukemia until approximately 500-600 days of age. This study will be completed in four years.

ENERGY, DEPARTMENT OF (Y01-CM2-0112)

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Extrapolation of Radiation Risk." This study will address a question of fundamental importance to the understanding of mechanisms of carcinogenesis and to risk estimation, namely, whether the natural incidence of cancer influences the susceptibility to induction by radiation or by other carcinogenic agents. The investigators will determine whether the natural incidence of specific cancers influences the susceptibility to induction by gamma radiation in two different strains of mice. The laboratory was able to expand the breeding stock somewhat faster than originally anticipated, and so Dr. Storer now has all 5,000 mice on hand for the experiment. All radiation exposures were completed by April 1, 1983. They are maintaining pedigree records on all the mice so that, in addition to obtaining cancer incidence by radiation dose, they will also be able to analyze the data for "litter effects" as well as "cage effects". Results are not expected to start becoming available until the third year of this project. This study will be completed in five years.

ENERGY, DEPARTMENT OF (Y01-CM2-0113)

This Interagency Agreement is for support of a new study at Oak Ridge National Laboratory entitled "Co-Carcinogenesis: Ionizing and Ultraviolet Radiation." This study will determine quantitatively the interactions of ultraviolet radiation and ionizing radiation (x-rays and fission neutrons) in terms of production of skin cancer in hairless mice. The persistence of initiated cells in skin will be investigated after exposure to doses of ionizing radiation that alone do not result in an increase in the incidence of skin cancer. These cells may be promoted later to cancer cells by other agents. For the skin cancer studies, the colony of SKH:hairless-1 mice has been built up and mice are being entered into the 4 different exposure groups. The exposure regimen for Experiment 1 has already been completed, that is, initial exposure to X rays followed by PUVA treatment. In recent studies on induction of skin cancer with UVR and 8-MOP plus near-UVR (PUVA), they have used the induction of pyrimidine dimers and DNA crosslinks, respectively, in basal cells as a form of molecular dosimetry. The measurement of these photoproducts has allowed them to determine the effective dose of carcinogen to reach the basal cell layer. This study will be completed in five years.

ENERGY, DEPARTMENT OF (Y01-COO-0320)

The primary objective of this Interagency Agreement is to measure the late effects of low doses of ionizing radiation in a large, relatively long-lived animal, the dog, to aid in assessing hazards and understanding mechanisms of radiation damage in man. These studies address two basic problems associated with exposure to low doses of ionizing radiation: 1) obtaining reliable data on biological responses at low dose rates when exposures are protracted, and 2) extrapolation of data from experimental animals to man. The studies use beagles to test whether the results from studies with shorter-lived, smaller and more radiation resistant rodents do, in fact, establish a constant radiation injury parameter, at low doses and dose rates, that is characteristic for all

ENERGY, DEPARTMENT OF (Y01-C00-0320) - (CONTINUED)

mammalian species. The data from these studies will help determine whether species differences in sensitivity (the dog is 3 times as sensitive as the mouse at high doses and dose rates) are a consideration in extrapolation to man. There were initially 257 Beagle dogs in this study and 154 are still alive. The initial control group of 180 dogs is maintained by ANL. Myelogenous leukemia occurs at total exposures greater than 2000 R, and at exposure rates of 5, 10, and 17 R, but not at 35 R/day. These limits are related to the fact that total doses above 1400 R given at 35 R/day are acutely lethal, and total exposures of over 2000 R are required to produce myelogenous leukemia in dogs irradiated continuously. Myelogenous leukemia from terminated as well as continuous irradiation occurs as the earliest detectable malignancy. Preliminary analysis of mortality data indicate 47% of control deaths have been due to malignancies while 63% of deaths in the irradiated dogs have been due to malignancies with increases in mammary carcinoma, neurofibrosarcoma and thyroid cancer. This study will be completed in about six years.

ENERGY, DEPARTMENT OF (Y01-C01-0711)

This Interagency Agreement supports research at Brookhaven National Laboratory which is designed to describe quantitatively, and to understand the explanations for, the shapes of dose response curves for genetic effects of ionizing radiation of different linear energy transfers (LET) at low doses or at high doses and low dose rates. The cells studied will be those of the stamen hairs of the plant Tradescantia, a well understood genetic system. The fraction of cells that change color from blue to pink as a result of irradiation can be measured with high precision at low doses (1 rad or less of gamma rays). No other biological system has this sensitivity. These experiments should give a firm experimental and theoretical base to the effects of low levels of environmental hazards in producing genetic, and presumably carcinogenic, effects in higher eukaryotic systems. The dose-response curve has been established for stamen hair somatic mutation in Tradescantia following exposure to ^{137}Cs gamma rays and confirmed the hypothesis of linearity below about 7.5 rad. The results of chronic and fractionated gamma exposures support the hypothesis that with low level radiation, the mechanism of action is exclusively that of the high LET component of the radiation. The duration of this study will be four years.

ENERGY, DEPARTMENT OF (Y01-C01-0712)

This Interagency Agreement is for support of research at Brookhaven National Laboratory. The experimental protocol is to irradiate different strains of mice with single doses, repeated doses and with a wide range of dose rates to determine: 1) the incidence of leukemia at low average dose rates; 2) the presence of preleukemic cells in the mice as a function of total dose and dose rate; 3) the number of preleukemic cells initiated by radiation; 4) the relative degree of "repair" following single, repeated, and continuous exposure; and 5) if there remains a fraction of the effects of low LET radiation that is nonreparable, or comparable to the "single hit" damage and effects seen with high LET radiation. The study is also designed to measure the incidence of

ENERGY, DEPARTMENT OF (Y01-C01-0712) - (CONTINUED)

preleukemic cells rather than waiting for the development of overt leukemia. Preleukemic cells can only be detected early by injection into a lethally or sublethally irradiated mouse, whereas the frankly leukemic cells grow later, but equally well in normal or irradiated hosts. An aim is to determine if preleukemic cells are present at very low average dose rates, where frank leukemia may not be detectable, perhaps because of statistical limitations. Most of the C57Bl/6 mice have been irradiated for Experiment I on lymphoma incidence, and most of the CBA/Ca mice have also been exposed for Experiment III on the incidence of radiation-induced leukemia. Dr. Cronkite has postponed the preleukemic assays until later as recommended by NCI reviewers. The duration of this study will be five years.

ENVIRONMENTAL PROTECTION AGENCY (Y01-CM2-0109)

Under this Interagency Agreement, via EPA Contract 68-01-4831, work has continued for the past year upon the development of a fully integrated Drug Information System (DIS). Several administrative modules of the DIS are now operative. These include an ordering and compound depletion module, and a name codes module. Systems that are approaching completion include the Inventory and the Pre-registry programs. The larger files such as chemistry and biology are scheduled for installation towards the end of 1983. All modules are designed to be automatically self-updating.

FLORIDA, UNIVERSITY OF (N01-CM9-7320)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of lung. Patients who are randomized to the nutrition intervention group received graded levels of nutritional support based on their nutritional status. In addition, this institution is evaluating the effect of nutritional intervention on the pharmacology of antineoplastic drugs. This contract is in its final year of follow-up and will expire in October 1983.

FLOW LABORATORIES, INC. (N01-CM0-7370)

This contract is concerned with the development of a practical scale-up procedure to manufacture 50 billion units of human fibroblast beta interferon. The interferon will be at a specific activity of 1-3 x 10^6 units/mg of protein. Because of difficulties in the scale-up procedure, this contract has been extended to allow for process refinement. At present the contractor has delivered approximately 3.5 billion units of interferon.

FLOW LABORATORIES, INC. (NO1-CM1-7398)

This resource contract provides the Division of Cancer Treatment with storage and distribution capabilities for he large volume of investigational and commercial drugs used in clinical trials. Approved orders for clinical drugs are packaged and shipped to destinations around the world. The contractor also provides a computerized inventory management system. This assures the proper rotation of stock, an adequate lead time to obtain new supplies of drugs and the prompt removal of expired materials.

FLOW LABORATORIES, INC. (NO1-CM2-7505)

This contractor furnishes the National Cancer Institute with facilities and services for the storage and distribution of synthetic chemicals, bulk chemical drugs and crystalline natural products. Samples are weighed, packaged and shipped to contract screening laboratories and also to various domestic and foreign research institutions. The contract also provides for the maintenance of accurate inventory records. This is an on-going operation and supports all of the DTP programs.

FOX CHASE CANCER CENTER (NO1-CMO-7330)

The capability for evaluating chemical compounds for radiation protecting properties is provided by this resource. Various physico-chemical parameters are determined on a large number of compounds representing a wide range of chemical structures. Compounds showing potential radioprotector characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiation protection using mammalian cell cultures. Compounds which appear to be superior to the standard - WR-2721 - will be evaluated in vivo, using mice and/or rats. The effect of the compound in modifying the response to radiation will be measured in three different tumor systems (from the DCT panel of mouse tumor screens as stated in the Treatment Linear Array for Radioprotectors), each measured by a separate endpoint. The endpoints will include: the regrowth delay of tumors, tumor cell survival and the modification of the radiation dose required for curing 50% of the tumors. All testing will be compared with the standard WR-2721. The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds.

This contract should provide new radioprotectors or leads in developing new types (classes) of radioprotective compounds. This contract is currently being recompeted.

FOX CHASE CANCER CENTER (NO1-CM9-7314)

This contract provides for the support of a clinical neutron therapy program at the University of Pennsylvania - Fox Chase Cancer Center using a DT generator developed under the direction of the University of Pennsylvania, in part through grant support from NCI. The addition to the Fox Chase Cancer Center to house the neutron therapy system was completed in October 1981. After several delays, the DT generator tube was shipped to Philadelphia in March 1982. Unexpected problems with both D-T generator tubes caused interruptions during 1982 and early 1983. Initial testing has been completed.

FRED HUTCHINSON CANCER CENTER (NO1-CMO-7445)

This Phase I study for the evaluation of toxicity and biological modifying effect of thymosin fraction 5 and alpha-1 is nearing completion. Patient accrual is complete and the laboratory testing was completed in June 1983. Fifty patients with non-oat-cell lung cancer and 17 patients with melanoma have been studied. No anti-tumor effect was detectable in this Phase I trial. No consistent biologic effect has been observed to date. The contract expired June 1983.

GENERAL SERVICES ADMINISTRATION (YO1-CM2-7519)

Under this contract, a method was implemented facilitating the input of chemical structures to produce, automatically, corresponding connection tables that are compatible with those presently used by the NCI (and other) systems.

GEORGE WASHINGTON UNIVERSITY (NO1-CMO-7446)

This contract is for the conduct of a Phase I/II evaluation of thymosin alpha-1 as an adjuvant to radiotherapy in patients with inoperable nonsmall-cell lung cancer. Early results suggested a benefit in terms of relapse free survival for patients treated with thymosin alpha-1 when compared to placebo. The accrual of additional patients by June 1983 will allow firmer conclusions to be drawn. Trends have been observed i in recovery of total T cell numbers, following radiotherapy in patients treated with thymosin alpha-1. This contract expired June 1983.

GEORGETOWN UNIVERSITY (NO1-CMO-7437)

A Phase I trial of human alpha interferon with 33 patients has been completed. The MTD for a sample dose has been determined to be greater than 60 x 10^6 units/m^2. Higher doses could not be given due to formulation problems. A stimulatory effect or natural killer cell and antibody dependent cell mediated toxicity was observed, most consistently at a dose of 60 million units/m^2. The contract continues with a Phase II trial evaluating 2 separate schedules for administration of human lymphoblastoid interferon (Wellferon®) in patients with metastatic melanoma. It is too early to evaluate results. This contract will expire October 31, 1983.

GEORGIA, UNIVERSITY OF (NO1-CM2-7401)

This contract with the Department of Pharmaceutics of the University of Georgia has the responsibility of performing shelf life evaluations of clinical drugs. This stability data is supplied to the Food and Drug Administration in support of the NCI's IND filings. The contractor monitors the stability of dosage forms at several storage temperatures. The testing involves the use of multiple analytical methods. The method most frequently used for assay of the stability samples is high performance liquid chromatography (HPLC). This contractor also has the responsibility of conducting reserve sample inspections as required by the Current Good Manufacturing Practices (CGMPs).

GEORGIA INSTITUTE OF TECHNOLOGY (NO1-CM2-7517)

This project is one of the three contracts which the objectives are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improve bio-availability by increasing aqueous solubility; (b) increase compound stability; (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles.

HARLAN INDUSTRIES (NO1-CMO-7362)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are effected in a closely controlled environment. All foundation colonies are rederived from NIH stock and maintained in associated flora isolators. All expansion colonies are maintained in the barrier environment. This contract terminated June 1983. A new competition is in progress for recompeting this effort.

HARLAN INDUSTRIES (NO1-CM5-0591)

This contract furnishes approximately 156,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or production colonies. This contract terminated April 1983. A new competition is in progress for recompeting this effort.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM2-3911)

This contact operates the Animal Production Area at the Frederick Cancer Research Facility. The contract operates as a Primary Genetic Center, Rederivation Center and Embryo Freezing Center. Strains are received from the NIH Repository and rederived for use at FCRF and distribution to other NCI contract activities. The bulk of the production on this contract is for supplying the animal needs of the researchers located at FCRF. Animals are also sent from FCRF to other NCI funded research activities.

HEALTH RESEARCH, INC. (NO1-AIO-2657)

This contract was initiated by the National Institute of Allergy and Infectious Diseases, and was funded by the Division of Cancer Treatment. The objectives of the contract are: (1) to develop standard preparations of polyriboinosinic-polycytidylic acid-poly-l-lysine carboxymethylcellulose (poly ICLC), and interferon inducer; (2) to characterize its physical properties; (3) to investigate reproducibility of poly ICLC made with components from various sources; (4) to study the effects of alterations in the formulation; (5) to assay the purity of components; (6) to investigate formulation without carboxymethyl-cellulose; (7) to evaluate various modified poly IC and poly ICL complexes for interferon induction capabilities and toxic effects; in in vitro cell culture systems, mice and sub-human primates; and (8) to develop in vitro and/or in vivo assays for interferon induction that would be simpler and less expensive than using Rhesus monkeys. This contract expired March 16, 1983.

HEALTH RESEARCH, INC. (NO1-CM9-7311)

The objective of this program is to evaluate in a Phase II study, photoradiation therapy as a means of local treatment of various malignancies in man. It is planned to determine its scope and limitations and especially to identify situations where it may offer a unique advantage over existing therapies as a treatment for patients who have failed other modalities. Photoradiation therapy involves irradiating hematoporphyrin derivative, which accumulates in malignant tissue, with appropriate laser light in the presence of oxygen. This process generates singlet oxygen, a highly toxic substance. A minimum of 25 patients per year will be studied.

HOWARD UNIVERSITY (NO1 CM2-7543)

This contract is part of a collaborative effort among three institutions to investigate the role of intraoperative radiotherapy in the treatment of intra-abdominal malignancies and to develop guidelines for intraoperative radiotherapy techniques for use by other investigators and by clinical cooperative groups in Phase II and III clinical trials. An optional task is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy. The collaborative effort will facilitate the evaluation of this new radiotherapeutic modality with respect to normal tissue tolerance, safety, and efficacy.

HYBRITECH, INC. (NO1-CM2-6010)

This contract performs coupling of chemotherapeutic drugs, toxins and radioisotopes to monoclonal antibodies directed against specific antigens found on human tumor cells. Appropriate tests are carried out on conjugates to demonstrate that the cytotoxic agent-antibody conjugates retain antigen-antibody specificity comparable to the unmodified antibody and cytotoxicity in excess of the non-derivatized cytotoxin. The contractor is required to scale up the appropriate conjugation procedure to provide sufficient quantities of a human use product for preclinical and preliminary clinical trials. Experiments have been initiated to couple adriamycin, ricin A chain, Ytterbium and Indium to three

HYBRITECH, INC. (NO1-CM2-6010) - (CONTINUED)

monoclonal antibodies: T101, an antibody directed against a human T-cell differentiation antigen, 9.2.27, an antibody directed against a human melanoma cell antigen and D-3, an antibody directed against a tumor specific guinea pig antigen.

IIT RESEARCH INSTITUTE (NO1-CM9-7213)

This contract provides assistance to the Drug Evaluation Branch staff in monitoring and evaluation of test data and the follow-up of materials demonstrating activity in the initial screening of new materials. The contractor participates with staff in the expediting of the scheduling of testing and the evaluation of data on those materials which are recommended for testing in the panel of antitumor test systems. Design of data files used as management tools by Developmental Therapeutics Program staff has been provided and the coordination of the data input to these files continues to be provided by the contractor. These files provide a tracking system for the status of drugs in the Linear Array from decision point 2A and beyond. This contract terminated May 31, 1983.

IIT RESEARCH INSTITUTE (NO1-CM9-7316)

The current level of testing in mice of potential anticancer agents under this contract is 23,000 L1210 equivalent tests per year. The contract provides for in vivo testing in the P388 leukemia pre-screen, for evaluation of materials in specified tumor panel models, for detailed evaluations requested by members of the NCI staff, and for characterizations and evaluations of tumor models as directed by the NCI Project Officer. All testing is carried out in accordance with the protocols of the NCI Developmental Therapeutics Program. Materials tested in the P388 leukemia pre-screen are new synthetic compounds and fractions of natural products provided by the NCI. Models of the conventional tumor panel now in use under this contract include the B16 melanocarcinoma, M5076, $CD8F_1$ mammary, Colon 38 and L1210 leukemia. Xenograft models include the colon, breast and lung models, with the colon and lung being phased out in accordance with the design of the new tumor panel.

ILLINOIS, UNIVERSITY OF (NO1-CM3-7513)

The objective of this contract is to do a worldwide survey of all the natural products literature, identifying new structures and reports of specific biological activity which may be related to cancer, and reports of biological activity of plant and animal extracts. This is a key contract for acquisition of new agents for the DTP screening program.

INDIANA UNIVERSITY FOUNDATION (NO1-CM1-7475)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CM1-7349)

The purpose of this contract is to provide data management and processing which enables simple and rapid retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols for treatment of brain tumors. This contract is currently being phased out with termination scheduled for April 1984.

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CM2-7510)

This contract supports the information needs of the Cancer Therapy Evaluation Program, DCT (CTEP, DCT) by providing comprehensive information management during the protocol review process, providing data on the objectives of both active and completed protocols, and providing data on the results of active and completed protocols. The system provides scientific and administrative information on: 1) treatment modalities (e.g. drugs, biological response modifiers, radiation, and surgery), 2) diseases, 3) protocols and 4) investigator teams.

In addition, a subcontracted effort to VSE Corporation provides for the maintenance and operation of the Drug Distribution and Protocol Monitoring System (DDPMS). The DDPMS is an automated procedure used to verify the accuracy of investigator drug requests, thus fulfilling our legal (FDA) requirements in that regard. Verified data is retained and forms a drug distribution history which is used to monitor protocol activity as clinical trials progress. The system also provides management information for the program, the cooperative study group and private organizations.

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CPO-1025)

An interdivisional transfer of funds to DCCP, Biometry Branch, to support statistical services for the Lung Cancer Study Group headed by Dr. Mitchell Gail. Monies support extramurally contracted data management with IMS.

INSTITUTE JULES BORDET (NO1-CMO-7350)

Materials collected in Northern Europe are screened in vivo against animal tumors in accordance with established NCI protocols. Materials that originated in the U.S. or other countries may be sent to this laboratory for testing. Testing is currently being conducted at a level of approximately 11,000 L1210 test equivalents per year. More detailed evaluation of drugs of interest to NCI is conducted upon request or agreement of the Project Officer.

INSTITUTE OF CANCER RESEARCH (NO1-CM1-7502)

The objectives of this project are to design, synthesize and evaluate novel compounds, both nitroimidazoles and other heterocycles, as potential radiosensitizers. Several classes of heterocycles with one or more electron withdrawing groups and bicyclic heterocycles have been synthesized and are in the process of evaluation. Experiments combining the clinically used misonidazole with other electron affinic agents and chemotherapeutic alkylating agents are also being evaluated. The tasks performed include measurements of electron affinity, lipophilicity, radiosensitization enhancement ratios and neurotoxicity. The focus of this project is to develop a radiosensitizer with less neurotoxicity than misonidazole. This contract is being phased out.

INSTITUTE OF CANCER RESEARCH (NO1-CM4-3736)

The objectives of this cost-sharing contract are to (1) study the biochemical and pharmacological bases for treatment failure or response; (2) acquire or synthesize potential anticancer agents designed to increase the therapeutic efficacy of known drugs; (3) evaluate new compounds synthesized by the contractor, or of interest to NCI, against human tumor xenografts and mouse tumors unique to the contractor; and (4) conduct toxicological studies to establish safe dosage levels and regimens for clinical evaluation of the drugs in the United Kingdom.

IOWA, THE UNIVERSITY OF (NO1-CMO-7303)

This contract provides services involving dosage form development and manufacture of investigational drugs for subsequent clinical evaluation. Compounds to be formulated are selected and provided by the National Cancer Institute. The contractor has produced primarily sterile freeze dried injectable products under this contract. However, the contractor has the capability to produce a wide variety of pharmaceutical dosage forms. The contractor is responsible for completing required analytical and safety tests on each product as well as monitoring the stability of the dosage form at recommended and elevated temperatures. All products are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators. This contract is currently being recompeted.

IOWA, THE UNIVERSITY OF (NO1-CMO-7334)

This contract provides capabilities to chemically characterize peptides, proteins and glycoproteins that may be used experimentally and/or clinically to modify tumor growth. Assay methods are developed to analyze the substance in bulk, dosage form and common pharmaceutical vehicles. Studies include determination of amino acid composition, molecular weight, isoelectric point, terminal sequence and development of suitable immunological measurement (radioimmunoasays, etc.) and suitable biological assays. This contract is currently being recompeted.

IOWA, UNIVERSITY OF (NO1-CM1-7476)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

IOWA, UNIVERSITY OF (NO1-CM9-7319)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status. This contract is in its final year of follow-up and will expire October 1983.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CMO-7338)

A major effort in breast cancer has been undertaken through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have received world-wide attention. The Istituto has recently shown an improved overall survival for premenopausal patients treated with CMF. They also recently reported that 12 months of CMF is no more effective than 6 months. A re-analysis of disease-free survival among postmenopausal patients showed a clear advantage for patients receiving an average >75% drug dose compared to those with <75% drug dose.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM2-2054)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs, and in the exchange of preclinical and clinical knowledge requisite for maximum progress in cancer therapy. The Japanese investigators survey the scientific literature in Japan and provide listings of relevant articles and abstracts to the Project Officer. The Office also handles exchange scientist programs between the U.S. and Japan. A small testing facility is maintained for the screening and further evaluation of selected new compounds. This contract is being recompeted.

JOHNS HOPKINS UNIVERSITY (NO1-CM2-7509)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contract is presently conducting one Phase I study with N-Methyl-formamide and has just started a new trial with Spiromustine.

JWK INTERNATIONAL, INC. (NO1-CM2-5602)

This project provides technical support services to the Office of the Director, DCT, as well as to the program areas of DCT in the performance of the planning and analytical tasks and general logistical support in the development of related or otherwise required documentation and conference support activities of the Division. This contract was awarded in December, 1981 for a three year period.

KANSAS, UNIVERSITY OF (NO1-CM0-7304)

This contract investigates approaches to resolve difficult dosage form development problems not amenable to usual solubilization or stabilization methods. This contractor has considerable expertise in the application of molecular complexes and reversible derivatives to improve solubility. The contractor also is responsible for pilot scale preparation and chemical analysis of the formulations developed under this contract. This contract is currently being recompeted.

KENTUCKY RESEARCH FOUNDATION, UNIVERSITY OF (NO1-CM0-7381)

Difficult dosage form development projects not amenable to the usual solubilization and/or stabilization approaches are assigned to this contractor. This contractor has particular expertise in the application of reversible derivatives (prodrugs) to improve drug solubility. Pilot batch preparation and chemical analysis of these novel formulations are carried out under this contract. This contract is currently being recompeted.

KING ANIMAL LABORATORIES (NO1-CM1-7499)

This procurement contract is designed to furnish 156,000 six-week old first-generation hybrid mice for Developmental Therapeutics Program contract studies. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

LABORATORY SUPPLY COMPANY (NO1-CM5-0577)

This contract furnishes approximately 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are being furnished from genetic centers and/or rodent production centers. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CM7-7165)

This primary genetic center has as its objective the development of associated foundation colonies of inbred rodents. Pedigree animals are derived via hysterectomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure culture of nonpathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to Rodent Production Centers, which in turn, produce large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies. This contract terminated June 1983. A new competition is in progress for recompeting this effort.

LITTON BIONETICS, INC. (NO1-CMO-7326)

The major objectives of this contract are to prepare and supply large quantities of concentrated primate and putative human type C RNA tumor viruses. This contract is being recompeted.

LITTON BIONETICS, INC. (NO1-CMO-7347)

The major objectives of this contract are: (1) the use of immunofluoresence and radioimmunoassays to screen human T cells and T cell lines for viral structural protein expression; (2) the use of ELISA assays to detect antibodies against a putative human virus in serum from leukemic patients and normal donors; (3) to test culture fluids from short-term and long-term cultured cells for the presence of viral DNA polymerase activity; (4) to test cultured cells for colony formation in semisolid media; and (5) to test sera from patients with T cell leukemia for antibodies to human type C RNA tumor virus (HTLV). This contract is being recompeted.

LITTON BIONETICS, INC. (NO1-CM1-5737)

This contract assists the intramural scientists in the Clinical Oncology Program in the storage and maintenance of laboratory animals. The facilities of the Clinical Center are extremely limited in the availability of space and personnel for laboratory animal handling, and this investigative resource is commonly available through a contract mechanism. The contract combines the animal holding and transport needs of all Branches in Clinical Oncology into one support contract. The contractor maintains, feeds, and transports the animals but does not conduct research. Tumored animal models are also provided.

LITTON BIONETICS, INC. (NO1-CM1-5808)

The objective of this contract is to confirm the stated biological properties of BRM preparations. Studies are carried out to evaluate and verify the potential of each BRM to reduce tumor growth in in vivo animal tumor models, to augment the immunizing capability in animal tumor models, to evaluate the mechanism of each BRM in in vitro tests, to determine an effective time and dose of administration and a nontoxic biologically effective dose. This contractor has developed in vivo antitumor assays with Meth A, Lewis Lung and LSTRA tumors, in vitro B-cell assays to measure augmentation of antibody response, and in vitro lymphokine/cytokine assays for macrophage activation factor, colony stimulating factors, and interleukin 1 and 2. The contractor has been involved in evaluation of MVE 2, MDP, Azimexon and N.137.

LITTON BIONETICS, INC. (NO1-CM2-5616)

The major objectives of this contract are: (1) to purify and supply factors that promote growth and differentiation of myelogenous leukemic cells and T cells; (2) to purify the envelope and internal structural proteins of type C RNA tumor viruses; (3) to prepare monoclonal antibodies against the purified structural proteins; (4) to provide cultured T and B cells from human cord blood, peripheral blood and leukemic cells; and (5) to prepare and supply radiolabeled cDNA and RNA probes from type C retroviruses.

LITTON BIONETICS (NO1-CM6-7067)

This contract supports Surgery Branch research by providing appropriate amounts of human and murine Interleukin-2 to conduct experiments. This research is directed toward developing new adoptive immunotherapies for the treatment of cancer using specifically sensitized lymphoid cells expanded in Interleukin-2 or using Interleukin-2 directly as an immune adjuvant.

MARYLAND, UNIVERSITY OF (NO1-CM2-7541)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor is presently conducting two Phase I studies with CBDCA and N-methylformide. Three other Phase I studies (AZQ, S Tumor and Leukemia; Aclacinomycin; and Homoharringtonine) have been concluded.

MARYLAND, UNIVERSITY OF (NO1-CM8-7223)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 32 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

MASON RESEARCH INSTITUTE (NO1-CM0-7325)

The purpose of this contract is to develop new in vivo tumor models with predictive value in selecting clinically effective drugs, to establish and maintain in serial transplantation human tumor cell lines in nude mice as assay systems, to validate human tumor xenografts as screening models, to develop other in vivo tumor models as indicated by Program needs, to maintain the assay developed, and to conduct special non-routine testing in assays other than the tumor panel upon specific request. The contract expired June 1983.

MASON RESEARCH INSTITUTE (NO1-CM8-7164)

This contract has as its major goal the maintenance of approximately 20,000 frozen tumor vials. This contractor furnishes needed tumors to the various DTP laboratories, as well as to other research institutions, both domestic and foreign. The tumors are supplied both in vivo and in vitro. This contract terminated July 1983. A new competition is in progress for recompeting this effort.

MASON RESEARCH INSTITUTE (NO1-CM9-7317)

Synthetics and materials of natural product origin are tested in the P388 leukemia mouse tumor test system as a pre-screen for activity in other in vivo test systems. Materials which are assigned to the tumor panel are tested in the B16 melanoma, the L1210 leukemia, and the M5076 mouse tumor test systems and the mammary human tumor xenograft test system. Additional test systems are available for special studies. Methodology research is conducted at the request of the Project Officer.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM1-7481)

This contract is part of a collaborative effort among three institutions to investigate the role of intraoperative radiotherapy in the treatment of intra-abdominal malignancies and to develop guidelines for intraoperative radiotherapy techniques for use by other investigators and by clinical cooperative groups in Phase II and III clinical trials. An optional task is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy. The collaborative effort will facilitate the evaluation of this new radiotherapeutic modality with respect to normal tissue tolerance, safety, and efficacy.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM2-7532)

This contract is part of a collaborative effort to improve treatment planning with neutrons and with charged particles (protons, helium ions, pi-mesons and heavy ions). In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, the collaborative working group is conducting a comparison of treatment plans for selected tumors in each anatomic site. Whenever possible, measurements in patients or phantoms will be made to check the accuracy of the treatment planning.

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CM2-7525)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

MATHTECH, INC. (NO1-CM9-7195)

The objective of this contract is to provide a clinical trials monitoring service for clinical trials conducted by the Phase I Working Group and the Biological Response Modifiers Program (BRMP). This service has two components: (a) to provide a central data management resource for the Investigational Drug Branch (IDB), the BRMP, and for the clinical investigators conducting these studies and (b) to provide a monitoring resource to meet the Food and Drug Administration (FDA) regulatory requirements and to complement the data management objectives. This contract expired June 1983, and is currently being recompeted.

MAYO FOUNDATION (NO1-CM2-7528)

This contract is part of a collaborative effort among three institutions to investigate the role of intraoperative radiotherapy in the treatment of intra-abdominal malignancies and to develop guidelines for intraoperative radiotherapy techniques for use by other investigators and by clinical cooperative groups in Phase II and III clinical trials. An optional task is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy. The collaborative effort will facilitate the evaluation of this new radiotherapeutic modality with respect to normal tissue tolerance, safety, and efficacy.

MAYO FOUNDATION (NO1-CMO-7419)

This is one of four contracts devoted to the application of a human tumor clonogenic assay to drug screening. Previous effort on this contract has resulted in the development of a quality controlled protocol useful for drug screening with a number of tumor types. Recent work has been confined to those which perform best in the assay: breast, colorectal, kidney, lung, and melanoma. A substantial amount of data has been gathered to support the validity and reproducibility of the assay for new drug screening. Initial screening results have been quite encouraging. A group of compounds which had been negative in the in vivo P388 leukemia system currently used as a pre-screen were tested in the clonogenic assay. Of these 10/77 showed significant activity. Several of these had outstanding in vitro activity and represent novel structural types. Current efforts on the contract are devoted to further drug screening and to a limited number of developmental studies aimed at further improving the protocol.

MAYO FOUNDATION (NO1-CM2-7539)

This contract provides support to continue the follow-up of GITSG patients accrued under contracts NO1-CM-43783, NO1-CM-43796 and NO1-CM-57033 (two major adjuvant studies in colorectal cancer and a variety of adjuvant and advanced disease studies in gastric and pancreatic carcinoma). Although these contracts expired, the patients on these studies are surviving longer than was predicted at the study's inception. This contract will be phased out in the latter part of 1983.

MAYO FOUNDATION (NO1-CM2-7548)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contract is presently conducting three Phase I trials with Tricyclic nucleotide, Henkel's Compound, and Bisantrene continuous infusion. Two combination Phase I studies (PALA/Alanosine in leukemia; VP-16/AMSA in leukemia) have been activated and one concluded during this period.

MAYO FOUNDATION (NO1-CM3-7601)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

MAYO FOUNDATION (NO1-CM9-7268)

This contract calls for a Phase II evaluation of therapies in advanced gastrointestinal cancer. Two important studies were performed during this last contract year. These were evaluating the soft agar cell cloning assay as a predictor for chemotherapy sensitivity in advanced colorectal cancer and a randomized trial of high dose Vitamin C in patients with advanced colorectal cancer who have received no prior radiation or chemotherapy. This contract expired in December 1982.

MELOY LABORATORIES, INC. (NO1-CM1-5757)

The purpose of this contract is to provide effective inventory, distribution and quality assurance confirmation for biological response modifiers. The contractor is responsible for receipt, dispensing, storage, distribution and inventory control of biological agents. Quality assurance evaluation involves specific assays to confirm sterility and assays to determine pyrogenicity and endotoxin levels. The contractor performs general safety tests for biological agents in compliance with Government regulations intended for clinical use and helps in the development of master files and IND's for biologics. Currently, the contractor provides for storage and distribution of approximately 30 different biologics. In the past year the contractor has performed general safety, pyrogenicity and other relevant testing on two lots of a monoclonal antibody preparation for use in clinical trials. The contractor has also worked with program in the development of the IND for submission to the Office of Biologics.

MELOY LABORATORIES, INC. (NO1-CM1-5813)

This resource contract provides for the production and purification of 5 billion units of human Type II (gamma) interferon from human buffy coats with a specific activity of at least 2×10^7 units per mg of protein. All delivirables have been received and meet conract specifications. This contract expired March 31, 1983.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM0-7337)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the testicle, esophagus, lung (small cell), gastric, sarcoma, urothelial, and head and neck. A minimum of 200 patients is studied with no less than 25 patients in any tumor type. The patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM1-7348)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM2-7546)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor is presently conducting Phase I trials with MMPR and Gallium Nitrate, and Fluoro-Ara-AMP in solid tumors, and with Homoharring-tonine in leukemias. A combination Phase I trial (PALA + MTX + 5FU) and a Phase I trial of high dose DDP in hypertonic saline, recently activated, are ongoing. Phase I trials with Homoharringtonine and Tricyclic neucleotide have recently been concluded.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM8-7224)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM8-7224)

standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 44 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

MIAMI, UNIVERSITY OF (NO1-CM9-7290)

Crude natural products with presumptive cytotoxicity in KB, P388, L1210, or astrocytoma cell culture, were followed toward development of anticancer potential in the appropriate in vitro test system, in support of the natural products program of DCT. Synthetic materials of particular interest and limited supply were also assayed by this contract. This contract was phased-out at reduced levels along with the natural products program it supported. This contract terminated February 28, 1983.

MICROBIOLOGICAL ASSOCIATES (NO1-CM9-7246)

This rodent production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of four strains of rodents.

MICROBIOLOGICAL ASSOCIATES (NO1-CM9-7287)

This contract functions in four major areas:

1. To operate and maintain a virus serum diagnostic laboratory. Serum samples are submitted from contract animal suppliers and testing laboratories. Approximately 90,000 virus tests are performed annually for this portion of the effort.

2. To test experimental tumors (animal and human) for viral contaminants. An estimated 1,000 tumors will be tested annually.

3. To perform an estimated 4,000 ELISA tests annually for the detection of Mouse Hepatitis Virus (MHV).

4. To produce vaccinia virus which is used for immunizing mice against infectious ectromelia. Approximately 100,000 doses of vaccine are produced annually.

MICROBIOLOGICAL ASSOCIATES (NO1-CMO-7369)

This contract resource assists in the measurement of leukocyte compatibility in clinical transfusions of leukopenic patients. The contractor performs several leukoagglutinin assays and lymphocyte cross-match studies in order to insure safe and reliable blood component transfusions. These tests are used in the daily management of the NCI leukopheresis program. In addition, the contractor operates a computerized serum storage bank for all patients at NCI which has accessioned over 20,000 samples. This contract was terminated in January, 1983.

MICHIGAN, UNIVERSITY OF (NO1-CMO-7405)

This contract conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, testicle, ovary, endometrium, cervix, head and neck, stomach, pancreas, and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. A minimum of 200 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherpy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

MICHIGAN, UNIVERSITY OF (NO1-CM8-7225)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 46 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (NO1-CM5-7009)

The major objective of this contract is the isolation of new antitumor agents from fermentations of marine, psychrophilic, and thermophilic organisms. These fermentations are screened against various enzymatic and other biochemical screens. Active products are isolated in sufficient quantities to be evaluated at the National Cancer Institute. In addition, various immunogen tests have been developed to evaluate the organisms and their metabolites as potential immunological stimulators specific for cancers. One chemotherapeutic agent from this contract, aclacinomycin, is in Phase II clinical trials in the U.S.A. Bactobolin has just passed Decision Network 2B and has been scheduled for toxicology. Deoxyspergualin has passed Decision Network 2A.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-7604)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

MIDWEST RESEARCH INSTITUTE (NO1-CM8-7234)

Midwest Research Institute is the smaller of the two contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological and clinical phases of the Developmental Therapeutics Program. The contractor determines the identity and purity of the compounds by appropriate methods. The contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (paper, thin layer, gas liquid and high pressure liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry and other methods as needed. Reports of the work performed by the contractor provide data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring. This contract is currently being recompeted.

MISSOURI, UNIVERSITY OF (NO1-CM2-7534)

This contract monitors the animal production by testing for the presence of Salmonella and Pseudomonas. Samples are received on a scheduled basis from the animal producers and approximately 9,000 fecal samples are tested per year. Contract NO1-CM9-7221 terminated in August, 1982 and a new contract, NO1-CM2-7534 was effective August 21, 1982 to continue the same workscope at Missouri.

MISSOURI, UNIVERSITY OF (NO1-CM8-7157)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. Special testing is provided on a priority basis when a suspected animal disease problem occurs at an animal supplier facility in a contract research facility. All testing is scheduled by the Project Officer.

MONSANTO RESEARCH CORPORATION (NO1-CM2-7516)

This service preparative contract provides for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. The effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

MONTEFIORE HOSPITAL (NO1-CM1-7474)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

MOUNT SINAI MEDICAL CENTER (NO1-AI2-2669)

This contract supports the International Bone Marrow Transplant Registry. The specific aims are 1) to maintain a statistical center for the collection, organization and analysis of clinical data provided by bone marrow transplant teams throughout the world; 2) to disseminate the results of clinically relevant analyses of pooled Registry data to bone marrow transplant teams, and to the medical profession for the earliest possible benefit to patients who might be helped by bone marrow transplantation treatment; and 3) to aid in designing, organizing and providing statistical support for controlled, cooperative clinical trials utilizing bone marrow for transplantation.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CB4-3879)

This contract is evaluating the usefulness of neuraminidase-treated allogeneic AML cells in acute myelocytic leukemia. Patients are randomized to receive either chemotherapy alone or chemoimmunotherapy (chemotherapy consists of cytosine arabinoside and daunorubicin). Patients have been studied both *in vivo* and *in vitro* with a variety of immunological testing parameters. This contract will expire in 1984.

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CM6-7096)

This contract, currently in phase out, is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. The contract expired June 30, 1983.

MURPHY BREEDING LABORATORIES (NO1-CM5-0579)

This contract furnishes approximately 156,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

MARYLAND, UNIVERSITY OF (NO1-CM2-7541)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor is presently conducting two Phase I studies with CBDCA and N-methylformide. Three other Phase I studies (AZQ, S Tumor and Leukemia; Aclacinomycin, and Homoharringtonine) have been concluded.

MARYLAND, UNIVERSITY OF (NO1-CM8-7223)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 32 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-3850)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals, standards for nomenclature used to identify stocks and strains of laboratory animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, and non-profit research institutions.

NAVY, DEPARTMENT OF (Y01-CM1-0200)

This contract is to cover the renovation and retrofitting of space in the Naval Hospital Center (NHBETH), Bethesda, Maryland, to be occupied by the NCI-Navy Medical Oncology Branch. The NCI-Navy Medical Oncology Branch began clinical responsibilities in July, 1981, and transferred officially to the NHBETH on October 1, 1981. Preliminary to this: (1) a "Memorandum of Understanding" was signed between the Surgeon General of the Navy and the Director, NIH, and the Director, NCI; (2) meetings were held between NCI-VA, other NCI officials, Navy staff and with the Navy consulting architects firm of Ellerbe, Dalton, Dalton, and Newport (see contract Y01-CM1-0201); (3) a draft agreement (prepared by Dr. J. Minna, NCI, and Capt. D. Pasquale, NNMC) governing the interaction of the NCI and NNMC was approved in principle by the Commanding Officer, NHBETH, and the Director, NCI. As part of this contract: (1) the NHBETH will provide a completely designed and functional clinical ward of 20 beds (ward 6 West) in their new hospital facility in Bethesda which was completed and had occupancy January, 1981; (2) two floors of temporary "swing space" in Building 1 (floors 4 and 5) for housing the outpatient clinic, offices, laboratories, and conference rooms; (3) four floors of "permanent" space in Building 8 (floors 3, 4, 5 and 6) to house the same functions; (4) the Navy retrofitting facility (Dept. of the Navy, Chesapeake Division, Naval Facilities Engineering Command, Washington Navy Yard), will supervise the retrofitting and renovation of the space on site and submit monthly reports as to the status of the work. The swing space in Building 1 has been completed and occupied while the renovation of Building 8 is almost complete. A change order is being prepared for the Building 8 renovation to accommodate a change in programatic requirements. The contract has been signed and approved by the Director, NCI, and the Commanding Officer, NHBETH, Bethesda, Maryland. The latest changes will require additional funds in FY 1983 for the negotiation of a new contract in order to complete the required renovations.

NAVY, DEPARTMENT OF (Y01-CM1-0201)

This contract is to cover the renovation and retrofitting of space in the Naval Hospital Center (NHBETH), Bethesda, Maryland, to be occupied by the NCI-Navy Medical Oncology Branch. The NCI-Navy Medical Oncology Branch began clinical responsibilities in July, 1981, and transferred officially to the NHBETH on October 1, 1981. Preliminary to this: (1) a "Memorandum of Understanding" was signed between the Surgeon General of the Navy and the Director, NIH, and the Director, NCI; (2) meetings were held between NCI-VA, other NCI officials, Navy staff and with the Navy consulting architects firm of Ellerbe, Dalton, Dalton, and Newport (see contract Y01-CM1-0201); (3) a draft agreement (prepared by Dr. J. Minna, NCI, and Capt. D. Pasquale, NNMC) governing the interaction of the NCI and NNMC was approved in principle by the Commanding Officer, NHBETH, and the Director, NCI. As part of this contract: (1) the NHBETH will provide a completely designed and functional clinical ward of 20 beds (ward 6 West) in their new hospital facility in Bethesda which was completed and had occupancy January, 1981; (2) two floors of temporary "swing space" in Building 1 (floors 4 and 5) for housing the outpatient clinic, offices, laboratories, and conference rooms; (3) four floors of "permanent" space in Building 8 (floors 3, 4, 5 and 6) to house the same functions; (4) the Navy retrofitting facility

NAVY, DEPARTMENT OF (YO1-CM1-0201) - (CONTINUED)

(Dept. of the Navy, Chesapeake Division, Naval Facilities Engineering Command, Washington Navy Yard), will supervise the retrofitting and renovation of the space on site and submit monthly reports as to the status of the work. The swing space in Building 1 has been completed and occupied while the renovation of Building 8 is almost complete. A change order is being prepared for the Building 8 renovation to accommodate a change in programatic requirements. The contract has been signed and approved by the Director, NCI, and the Commanding Officer, NHBETH, Bethesda, Maryland. The latest changes will require additional funds in FY 1983 for the negotiation of a new contract in order to complete the required renovations.

NAVAL HOSPITAL BETHESDA (NHBETH) (YO1-CM2-0200)

This Interagency Agreement supports the NCI-Navy Medical Oncology Branch, a Clinical Oncology Program branch which collaborates with the Hematology/ Oncology Branch of the National Naval Medical Center. Clinical trials and related laboratory investigations have been developed and are being supported through this agreement. Clinically, the collaboration will enhance patient care at the NHBETH for patients with malignant disease through combined NCI-Navy clinical protocols. The laboratory portion of the unit focuses its study on human tumor cell biology, whose results will hopefully be of direct benefit to the treatment of the NHBETH-NCI patients.

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CM1-7473)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CM9-7321)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status. This contract is in its final year of follow-up and will expire in October 1983.

NORTH CAROLINA, UNIVERSITY OF (NO1-CM1-7471)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CM8-7154)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 74 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

NORTHWESTERN UNIVERSITY (NO1-CM1-7363)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The contractor carries out the service by performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM2-7540)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor has conducted two Phase I trials since January 1983 (Henkel's compound and Dihydroazacytidine). A third Phase I trial with Fluoro-Ara-AMP has just started.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM3-7598)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established antitumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM8-7161)

The general objectives of the project are concerned with the acquisition of pharmacokinetic data in patients on new and established antitumor agents. Coupled with information on the quantitative toxicology and the clinical response to the drug, the pharmacokinetic studies are used to design an optimum therapeutic dosage regimen. A sensitive, selective, reversed phase HPLC assay was developed for the determination of plasma and urine levels of the antitumor agent DHAD (Mitoxantrone NSC-279836). This method was applied to the determination of the plasma concentrations of DHAD in 25 adult cancer patients receiving 6-12 mg/m^2 as a rapid intravenous (3-32 min) infusion during a Phase I clinical trial. The plasma concentration-time data were NONLIN computer fitted to a three compartment open model with first-order elimination from the central compartment using the equations for a short intravenous infusion. The initial disposition phases were very short ($t_{1/2}$ = 1.78 min and $t_{1/2}$ = 15.7 min) and followed by an apparent terminal elimination (gamma) half-life of 3.01 hours. The apparent volume of distribution of the central compartment was approximately 7% of body weight (V_1 = 2.83 L/m^2). The steady state volume of distribution was approximately 64% of body weight (Vd_{ss} = 25.9 L/m^2), a value similar to that of total body water. The

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM8-7161) - (CONTINUED)

large apparent volume of the peripheral compartment Vd = 99.7 ± 55.9 L/m^2, (mean ± S.D.), indicates that DHAD is highly bound to the tissues. Of the microscopic rate constants, k_{31} had the smallest value, suggesting that the release of DHAD from deep tissue binding sites is rate-determining. The mean plasma total body clearance was 0.34 ± 0.13 $L/min/m^2$. A comparison of the DHAD pharmacokinetic parameters for the group of patients receiving 6 mg/m^2 (n = 16) with those receiving 12 mg/m^2 (n = 7) did not show any dose dependency of total body clearance, terminal half-life or other parameters for this limited dose range. Evidence for the presence of a fourth exponential phase (delta phase) in the pateint plasma concentration-time profiles was obtained. No evidence was obtained for the presence of metabolites in patient urine, plasma or red blood cells. However, extracts of bile samples obtained from rats and dogs showed the presence of one major metabolite, 2 or 3 minor components, and unchanged DHAD. The major biliary metabolite has properties identical to that of 8,11-dihydroxyhexahydronaphtho[2,3-f]quinoxaline-7,12-dione obtained synthetically from DHAD.

ONTARIO CANCER INSTITUTE (NO1-CM9-7267)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status. This contract is in its final year of follow-up and will expire October 1983.

ORKAND CORPORATION (NO1-CM3-6010)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programing expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the Clinical Branches of the Division of Cancer Treatment.

PAN AMERICAN HEALTH ORGANIZATION (NO1-CM2-7391)

The Collaborative Cancer Treatment Research Program of paired U.S. - Latin American investigators is currently concentrating efforts towards Phase II studies in diseases such as gastric carcinoma, vulvar, penile and cervical squamous cell carcinomas very prevalent in Latin American Countries expected to generate important unavailable data in clinical oncology. Lesser numbers of Phase III studies are left at this point. An adjuvant study in osteosarcoma involving multiple centers is being prepared.

PAPANICOLAOU CANCER RESEARCH INSTITUTE (NO1-CM8-7230)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. Special testing is provided on a priority basis when a suspected animal disease problem occurs at an animal supplier facility in a contract research laboratory. All testing is scheduled by the Project Officer.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM2-7529)

This contract is part of a collaborative effort to improve treatment planning with neutrons and with charged particles (protons, helium ions, pi-mesons and heavy ions). In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, the collaborative working group is conducting a comparison of treatment plans for selected tumors in each anatomic site. Whenever possible, measurements in patients or phantoms will be made to check the accuracy of the treatment planning.

PHARM-ECO LABORATORIES, INC. (NO1-CM1-7487)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. About 50% of the effort of this contract is devoted to the preparation of large quantities of material, in the multikilogram range.

POLYSCIENCES, INC. (NO1-CM3-7557)

This service preparative contract provides for the large-scale extraction of various plants and isolation and purification of the active materials for preclinical development and clinical trials. Cost and yield data are obtained on these processes. In the case of those plant processes which will be used again, process development optimization studies are conducted.

PROGRAM RESOURCES, INC. (NO1-CM2-3910)

The Government-owned contractor-operated NCI-Frederick Cancer Research Facility (NCI-FCRF) serves as a multi-faceted contract involving the following activities for the DCT:

Fermentation Program

The DCT supports a fermentation pilot plant facility at the NCI-FCRF for the large-scale production and isolation of microbial products of interest to the Developmental Therapeutics and Biological Response Modifiers Programs. Facilities are also available for the production of other natural products. Large-

PROGRAM RESOURCES, INC. (NO1-CM2-3910)

scale production is being evaluated to produce human monoclonal antibody which is potentially for human clinical use. Large quantities of Interleukin-III and other biologicals were produced for BRMP research projects during the last contract year. Kilogram quantities of toyocamycin have been produced for use as a precursor for the tricyclic nucleotide which is in Phase II clinical trials. Fermentation and development is underway to increase yields of largomycin FII. This compound has passed DN 2A. A novel antineoplastic agent has come from the earlier research effort and larger amounts of material will be produced to continue NCI evaluation.

RESEARCH TRIANGLE INSTITUTE (NO1-CM0-7352)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

RESEARCH TRIANGLE INSTITUTE (NO1-CM2-7515)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at the Institute or acquired from other sources, are analyzed for purity and identity by autography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

RESEARCH TRIANGLE INSTITUTE (NO1-CM3-7599)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established antitumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

ROXANE LABORATORIES, INC. (NO1-CM6-7053)

This resource contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms are manufactured in conformity to FDA Current Good Manufacturing Practices. These dosage forms are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

SASCO (NO1-CM9-0164)

This procurement contract provides for the supply of 156,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

SIDNEY FARBER CANCER INSTITUTE (NO1-CM5-7035)

This contract is designed to support prospective randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. This contract is due to expire in 1983 and will not be recompeted.

SIMONSEN LABORATORIES (NO1-CM5-0578)

This contract furnishes approximately 156,000 six-week old B6C3F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

SIMONSEN LABORATORIES (NO1-CM7-7166)

The contractor maintains a primary genetic center of inbred strains of rodents. Small quantitites of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies from pedigreed expansion colonies. All pedigreed foundation colonies are maintained in associated flora isolators. This contract terminated June 1983. A new competition is in progress for recompeting this effort.

SIMONSEN LABORATORIES (NO1-CM9-7247)

This contract provides for the maintenance of a rodent production center. This produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirement for specific investigations. This contract furnishes breeding animals for large-scale production colonies. The breeding stock is received from the primary genetic centers.

SISA, INC. (NO1-CMO-7354)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CM2-5600)

This contract is for the production of murine monoclonal antibodies directed against human cytokines. The contractor develops appropriate immunizing protocols to confirm the immunogenicity of the human cytokine in mice, and produces and isolates individual hybridoma clones secreting monoclonal antibody. Appropriate radioimmune assays are developed for screening individual hybridoma clones for antibody reactivity and ability of monoclonal antibody to specifically bind to and inhibit each cytokine. The contractor provides anti-cytokine secreting hybridomas and semi-purified immunoglobulin derived from the various hybridomas. The contractor has initiated studies for the development of hybridomas secreting monoclonal antibodies against human IL-2, and human gamma interferon and will soon undertake development of monoclonal antibodies against murine tumor necrosis factor.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CMO-7435)

This contract was awarded to conduct a Phase I/II clinical trial of human lymphoblastoid interferon (Wellferon®) in patients with multiple myeloma (in conjunction with Duke University and UCLA) and in patients with colon cancer. This contract will expire October 31, 1983.

SMALL BUSINESS ADMINISTRATION (BIOTECH) (NO1-CM3-7609)

The purpose of this contract is to provide supportive services in small animal studies, immunology, and tissue culture. At the present time, these functions include primarily the following: (1) detailed karyotypic analysis, including Giemsa banding, of a variety of monolayer and suspension cultured cells; (2) evaluation of tumorigenicity of various cultured cells by inoculation into nude mice; (3) testing the tumorigenic potential of selected primate retroviruses; (4) preparing small quantities of selected (frequently cloned) cells and retroviruses; and (5) testing various tissue cultured cell specimens for mycoplasma contamination.

SMALL BUSINESS ADMINISTRATION (MAXIMA CORPORATION) (NO1-CM1-7400)

The objective of this small business contract is to perform a variety of computer searchers such as full structure searches, substructure searches and data item searches in support of our program. The contractor utilizes several data bases such as NCI, Darc, Questel, NLM, and Dialog. Another newly added task under this contract is the development of chemical synonyms for compounds of interest.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM1-7521)

Social & Scientific Systems, Inc. provides technical assistance and support services in the area of investigational drug regulations and clinical research. Information is gathered and assembled for the preparation of Investigational New Drug Applications (IND's). This includes screening information, animal toxicology, chemistry, bibliographic information, drug labeling and the clinical protocol. This information is submitted to the Food and Drug Administration, and an IND is awarded. This contractor then maintains the files by providing in-depth tracking of drugs and amending IND information as necessary. SSS also provides drug distribution monitoring assistance and is involved with the preparation of IND annual reports, the establishment of drug master files, the distribution of clinical brochures, and the dissemination of adverse drug reaction information.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM2-5606)

This contractor provides support services for conference management and associated general logistical activities for the Cancer Therapy Evaluation Program. Logistics support includes various technical and clerical tasks ranging from report design and preparation to routine typing. Conference support includes both pre- and post-conference management activities necessary to successfully conduct large, as well as, small meetings and provide the results thereof to the biomedical research community.

SOUTH FLORIDA, UNIVERSITY OF (NO1-CM8-7220)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 75 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

SOUTHERN ANIMAL FARMS (NO1-CM5-0599)

This contract furnishes approximately 156,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated May 1983. A new competition is in progress for recompeting this effort.

SOUTHERN ANIMAL FARMS (NO1-CM9-7245)

This contract provides for the maintenance of a Rodent Production Center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM0-7421)

This contract supports a Phase I trial of the anti-T cell antibody, T101, in the treatment of patients with T cell malignancies (T cell CLL, Sezary Syndrome, Mycosis Fungoides and T cell ALL). Provisions are also included for immune absorption of free circulating antigen and antigen-antibody complexes prior to therapy. Other objectives include the ascertainment of T101 binding to the T65 T cell antigen in vivo, the determination of the serum half-life of T101, the determination of the degree of anti-mouse antibody formates and a determination of the degree of antigenic modulation. Patient accrual in this study is underway.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM2-7483)

This contract is conducting Phase II studies of photoradiation therapy for local malignancies. Efforts will be primarily focused on lung lesions with a minimum of 25 patients per year being studied. It is planned to determine its scope and limitations and especially to identify situations where it may offer a unique advantage over existing therapies.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM3-7600)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

SOUTHERN RESEARCH INSTITUTE (NO1-CM0-7260)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

SOUTHERN RESEARCH INSTITUTE (NO1-CM3-7552)

This contract was awarded to the Southern Research Institute in February 1983 following recompetition of contract NO1-CM0-7302. Utilizing a variety of murine leukemia and solid tumor models, the contractor (1) evaluates the antitumor activity of congeners or prodrugs of known active drugs or relatively new classes of agents; (2) evaluates the activity of purified natural products; and (3) conducts special detailed drug evaluation studies on compounds identified as candidates for development to clinical trial. In the latter studies the contractor may evaluate the activity of a formulated product, or determine whether an agent is schedule-dependent, more active in combination with another compound or active against a tumor with acquired resistance to a clinical drug.

SOUTHERN RESEARCH INSTITUTE (NO1-CM3-7597)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established antitumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

SOUTHERN RESEARCH INSTITUTE (NO1-CM9-7309)

This multifaceted project is designed to provide DCT with a flexible instrument for the rapid conduct of Program-directed developmental and applied tasks related to pre-clinical therapy. Principal tasks include (1) in vivo screening against transplantable animal tumors and human tumor xenografts; (2) detailed evaluation of drugs in development to NCI-sponsored clinical trial to determine their optimum conditions of usage; (3) application of fundamental biological principles to the development of new and improved laboratory models for the

SOUTHERN RESEARCH INSTITUTE (NO1-CM9-7309) - (CONTINUED)

discovery of more effective anticancer agents; and (4) quality control studies leading to the refinement of testing protocols. This project is currently undergoing competition with each of the four above-mentioned tasks to be awarded as separate contracts.

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CMO-7356)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

SRI INTERNATIONAL (NO1-CMO-7351)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

SRI INTERNATIONAL (NO1-CM1-7485)

The objective of this contract between NCI, SRI International and Stanford University is the synthesis and biological evaluation of novel radiosensitizers. The primary focus of the work is the identification of leads other than 2-nitroimidazoles. Several new types of compounds have been investigated including quinoxaline, 1,4-dioxides, pyridine N-oxides, benzotriazoles and glutathione depleters (diethylmaleate analogs). N-oxides of pyridine, pyrazine and quinoxaline look promising based on both *in vitro* and preliminary *in vivo* evaluations. These will be developed further.

SRI INTERNATIONAL (NO1-CM2-7560)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute.

SRI INTERNATIONAL (NO1-CM3-7605)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

STANFORD UNIVERSITY (NO1-CB1-4337)

The principal aim of this contract is to carry out research and laboratory evaluation of contrast enhancement of ultrasound images of tumors, organs, and vascular systems by the intravenous injection of encapsulated microbubbles. The studies are conducted in ultrasound phantoms and in tumor-bearing rabbits. This contract expired May 1983, but a one-year extension at no additional cost is being sought to continue the testing activity.

STANFORD UNIVERSITY (NO1-CM1-7480)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

STARKS ASSOCIATES, INC. (NO1-CM0-7357)

The Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

The Task Order Contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions. This contract is being recompeted.

STARKS ASSOCIATES, INC. (NO1-CM1-7374)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 50% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities and/or quality needed from the original supplier or on the open market. Preparations vary in quantity from gram to multikilogram scale.

STARKS ASSOCIATES, INC. (NO1-CM8-7206)

This contract is in support of the Drug Synthesis and Chemistry Branch's, fundamental responsibility to acquire selected novel synthetic compounds for evaluation as potential anticancer agents the initial step in the National Cancer Institute's Linear Array for drug development. The major focus of this contract is the active solicitation, acquisition documentation and management of the flow of approximately 10,000 compounds per year of diverse structural types. These compounds are selected by the Drug Synthesis and Chemistry Branch from a much larger pool of compounds provided through this contract in quantities adequate for the primary anticancer screen. This contract also acquires a significant proportion of the larger samples needed for secondary screening (Tumor Panel) of the many new leads that are identified.

STATE UNIVERSITY OF NEW YORK (NO1-CM2-7570)

This project is one of the three contracts which the objectives are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improve bio-availability by increasing aqueous solubility (b) increase compound stability; (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles.

TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES (NO1-CM1-7472)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, are used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated. This contract is currently being phased out because of budget cuts.

TENNESSEE, UNIVERSITY OF (NO1-CM3-7607)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

TEXAS, UNIVERSITY OF (NO1-CB8-4248)

The objective of this contract is to evaluate, in a controlled clinical study, the effect of intralesional injection of BCG crude cell walls on canine breast carcinoma. Dogs clinically free of detectable metastatic disease are randomly assigned to intralesional immunotherapy prior to surgery. The BCG crude cell walls are provided by the NCI. Tumor regression, tumor recurrence, disease-free interval, and survival data from the two are compared. Selected assays of humoral and cellular immunity are performed and results correlated with clinical course. Examination of serial serum specimens is performed to correlate serum interferon levels with treatment course and disease. Approximately 200 dogs per year have been entered in the study. This contract expired June 1983.

TEXAS A & M UNIVERSITY (NO1-CM3-7536)

This contract monitors the genetic purity of the strains produced at the Genetic Centers and Rodent Production Centers. The testing is done by checking biochemical markers, and animals are sent for monitoring on a weekly basis scheduled by the Project Officer.

TEXAS, UNIVERSITY OF (NO1-CM1-7524)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER (NO1-CM2-7542)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. The contractor has two ongoing Phase I trials with Fluoro-Ara-AMP and Echinomycin. A third study with 7-OMEN is scheduled to begin in the summer 1983.

TEXAS, UNIVERSITY OF, MEDICAL BRANCH (NO1-AI0-2659)

The Biological Response Modifiers Program funded this project entitled "Antisera to Immune Interferons" in Fiscal Year 1980. The contract is administered by NIAID. The objective of the contract is to produce antisera to human immune (type II) and mouse immune (type II) interferons in the required quantity to be used as NIH reference agents. This contract expired December 31, 1982.

TEXAS, UNIVERSITY OF, MEDICAL BRANCH (NO1-CM8-7221)

A member of the Head and Neck Contracts Program which is a collaborative group of six (6) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy versus a regimen consisting of induction chemotherapy followed by the standard regimen, versus induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-Platinum and Bleomycin. Cis-Platinum alone is utilized as the maintenance chemotherapy. A total of four hundred and sixty-two (462) patients have been randomized to the study between October 1978 and April 1982. This group had 31 patients on this study. Accrual of patients was terminated on April 30, 1982. Follow-up under the contract will continue until April 1984.

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL & TUMOR INSTITUTE) (NO1-CM2-7531)

This contract is part of a collaborative effort to improve treatment planning with neutrons and with charged particles (protons, helium, ions, pi-mesons and heavy ions). In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, the collaborative working group is conducting a comparison of treatment plans for selected tumors in each anatomic site. Whenever possible, measurements in patients or phantoms will be made to check the accuracy of the treatment planning.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL & TUMOR INSTITUTE) (NO1-CM3-7602)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established anti-tumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL & TUMOR INSTITUTE) (NO1-CM0-7406)

This contract conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, testicle, ovary, endometrium, cervix, head and neck, stomach, pancreas, and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. A minimum of 200 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL & TUMOR INSTITUTE) (NO1-CM2-7550)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor is presently conducting two Phase I trials with Fluoro-Ara-AMP and Tiazofurin and has recently closed a trial with Tricyclic nucleotide. Several other trials (Homoharringtonine, Spirogermanium, and Procarbazine) were carried over from a predecessor contract.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL & TUMOR INSTITUTE) (NO1-CM7-7149)

This contract provides follow-up support for patients included in three adjuvant clinical trials for non-small cell lung cancer through the Lung Cancer Study Group. Patients continue to be followed at regular intervals as determined by the protocols. This contract expired June 1983.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL & TUMOR INSTITUTE) (NO1-CM9-7277)

This contract was extended to support the completion of two Phase II studies. The Phase II studies are conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of lung, breast and colon. This extension expired May 1983.

UPJOHN COMPANY (NO1-CM0-7380)

This contract has as its primary objective the development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes. The contract includes the preparation of fermentation beers, isolation, purification, characterization and production of the active component. *In vitro* screening methods developed are used for assays on fractionation samples and for primary screening. Leads developed from these screens are being tested in-house in the leukemia *in vivo* screen and actives are given top priority for chemical fractionation. This contract was completed in December 1982.

UTAH, UNIVERSITY OF (NO1-CM1-7523)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Bureau of Radiologic Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

UTAH, UNIVERSITY OF (NO1-CO2-3917)

This is a new research contract for "Assessment of Leukemia and Thyroid Disease in Relation to Fallout in Utah". The purpose of this project is to conduct a detailed re-assessment of the possible long-term effects of radioactive fallout resulting from atmospheric weapons testing at the Nevada Test Site between 1950 and 1962. This contract includes 1) a study of milk consumption patterns in relation to milk sources and fallout exposures, 2) a case-control study of thyroid cancer in Utah, 3) a cohort study of malignant and benign thyroid disease, 4) a case-control study of leukemia in Utah, and 5) a cohort mortality study of leukemia in Utah. This large research project appears to be adequately directed and coordinated by Dr. Lyon. Very good progress has been made on all

UTAH, UNIVERSITY OF (NO1-CO2-3917) - (CONTINUED)

aspects of the project except the milk survey. Dr. Lyon and his staff have recently redirected their efforts away from trying to negotiate a subcontract with Dr. Kleinschuster and toward collecting and analyzing the milk survey data themselves. This should allow them to keep on schedule and have this survey completed by December 31, 1983. Representatives of DOD and DOE have been apprised of the current status of this project. This project will be completed in five years.

VANDERBILT UNIVERSITY MEDICAL CENTER (NO1-CM7-7122)

This contract provides follow-up support for patients included in four adjuvant clinical trials for non-small cell lung cancer through the Lung Cancer Study Group. Patients continue to be followed at regular intervals as determined by the protocols. This contract expired June 1983.

VERMONT, UNIVERSITY OF (NO1-CM2-7547)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations of regimens mutually agreed upon. This contractor conducted two Phase I trials with Homoharringtonine and Dihydroazacytidine. Homoharringtonine was carried over from the predecessor contract. This contractor is scheduled to initiate a Phase I trial with Tiazofurin in the immediate future and a trial with Spiromustine later this spring.

VERMONT, UNIVERSITY OF (NO1-CM3-7606)

This Master Agreement for preclinical pharmacology task orders was established to conduct preclinical studies of the pharmacology of new or established antitumor agents. Most of the drugs to be studied will be under development and have passed Decision Point 2B in the DCT Decision Network, i.e. scheduled to begin preclinical toxicology. This task order mechanism will enable the prompt solicitation and award of projects to be conducted in this area from a pool of highly qualified contractors.

VETERANS ADMINISTATION MEDICAL CENTER (VAMC) (YO1-CM3-0256)

This Interagency Agreement provides for the space and care of small animals required for the research purposes of the NCI-Navy Medical Oncology Branch. Space is provided to house conventional mice, nude mice, rats and rabbits. This agreement also includes technical services on an occasional basis by VAMC animal room technicians. These services are the embedding, cutting and staining of tissue sections.

VSE CORPORATION (NO1-CMO-7251)

This contract was awarded as the result of a competition held in calendar year 1979. Data processing services are provided to the Developmental Therapeutics Program by this contract. The scope of work includes (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch to both staff and the suppliers of the compounds being tested; (2) documenting all computer programs and contractor's procedures for data handling and running computer programs; (3) maintaining computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) modifying the existing data system so that data from new antitumor systems can be handled (e.g., the Human Tumor Stem Cell Cloning Assay, and the Astrocytoma In Vitro Assay); (5) refining the data collection methods; (6) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data; (7) providing output for statistical evaluation of test systems and evaluation of test system parameters; and (8) participating in scientific meetings.

WARNER-LAMBERT COMPANY (NO1-CM1-7491)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. About 30% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

WARNER-LAMBERT COMPANY (NO1-CM3-7285)

This No-Cost contract to develop and market AZQ as an antitumor agent was recently initiated. Its purpose is to facilitate development of the drug to the NDA stage.

WARNER-LAMBERT COMPANY (NO1-CM3-7614)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates from various parts of the world and fermented under a bevy of environmental and stress situations; (2) an in vitro tissue culture assay laboratory which assists in prescreening fermentation broths for cytotoxicity and is used to help assay chemical fractions, fermentation improvement samples and large pilot plant batches more quickly; (3) the isolation work required to obtain the active component from the confirmed active beers; (4) the production of large quantities of antineoplastic agents approved for clinical trials. One compound has passed DN 2A and is currently under development.

WASHINGTON, UNIVERSITY OF (NO1-CB8-4247)

The study objective was completed by better determining the conditions and operative mechanisms for maximizing the therapeutic effect of adoptive cellular immunotherapy used alone or in combination with chemotherapy in the treatment of tumors of C57BL/6, BALB/c and CB6F1 (BALB/c female x C57BL/6 male) mice. The approach was to utilize, in adoptive cellular immunotherapy experiments, cells immunized both in vitro and in vivo. Studies were undertaken to characterize the effector cells responsible for tumor therapy as well as cells which suppress the therapeutic effect. The role of the antigens for the major histocompatibility complex in sensitization to tumor associated antigens and in the generation of cells therapeutically effective against established syngeneic tumors was explored. Much of the data derived from the study has been published in 12 articles in refereed journals and 8 review chapters. This contract expired January 1983.

WASHINGTON, UNIVERSITY OF (NO1-CM9-7282)

This contract provides a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support a clinical neutron therapy research program at the University of Washington. The fabrication of the cyclotron and other components of the neutron therapy system has been completed. All components and accessories have arrived in Seattle and are being installed. Construction is underway and the facility should be completed in mid 1983. It is expected that the facility will be fully operational in October 1983.

WAYNE STATE UNIVERSITY (NO1-CM0-7404)

The Contractor conducts Phase II/III studies in patients with solid disseminated tumors. A minimum of 200 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator. This group continues to make important contributions to the development of new drugs.

WAYNE STATE UNIVERSITY (NO1-CM2-7551)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor is conducting two Phase I trials with Tricyclic nucleotide and Echinomycin. The Tricyclic nucleotide trial was carried over from a prior contract. In 1983, this contractor is scheduled to start two trials with Acodazole and Spiromustine.

WISCONSIN, UNIVERSITY OF (NO1-CM0-7434)

This contract was awarded to conduct a Phase I/II clinical trial of human lymphoblastoid interferon (Wellferon®). The Phase I study is using induction of the enzyme 2' 5' oligoadenylate synthetase as a determination of a maximum biologically effective dose. This dose will then be used in a Phase II trial in patients with non-oat-cell lung cancer. This contract will expire October 1983.

WISCONSIN, UNIVERSITY OF (NO1-CM2-7549)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon. This contractor conducted two Phase I trials with CBDCA and Misoindazole/Cytoxan. A trial with Tiazofurin is just starting and a second trial with Acodazole is scheduled to begin in the near future.

WISCONSIN MEDICAL COLLEGE (NO1-AI0-2658)

The Biological Response Modifiers Program funded this project entitled "Immune Interferon Standards" in Fiscal Year 1980. The contract is administered by NIAID. The objective of the contract is to provide NIH with 1,200 ampules of (1) a suitably stable, potent, freeze-dried immune interferon prepared from human lymphocytes and (2) an equal number of a similarly suitable preparation prepared from mouse lymphocytes for use as standard reference reagents. Testing is in progress at multiple laboratories to evaluate the interferon preparation by a standardized protocol. This contract will expire August 1983.

YALE UNIVERSITY (NO1-CB7-4191)

This contract evaluated the therapeutic efficacy of intratumoral BCG prior to surgery for carcinoma of the lung. Patients with potentially resectable lung carcinoma are randomly assigned to either treatment with intratumoral BCG two weeks prior to surgery or to surgery alone. Survival and disease-free interval are being analyzed. This contract expired February 1983.

YAMANOUCHI PHARMACEUTICAL CO., LTD. (NO1-CM9-7307)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the Division of Cancer Treatment. The contractor is responsible for conformity to U.S. FDA Current Good Manufacturing Practices and for completing all required analytical testing on each product prepared. All products are packaged, labeled and shippped to the National Cancer Institute for subsequent redistribution to clinical investigators.

ANNUAL REPORT - SCIENTIFIC INFORMATION BRANCH 1982 - 1983

The Scientific Information Branch was established in 1980 to provide overall management and direction to the publications of the DCT and its information gathering and reporting services. The SIB is located in the Office of the Director, DCT, NCI and contains two major components: the Publications Section and the Literature Research Section. Its activities can be divided into three major areas: the dissemination of scientific information in Cancer Treatment Reports, Cancer Treatment Symposia, and PDQ, the NCI's new cancer treatment information database; the preparation of information surveys, reports and reviews for the Director and the scientific staff of the DCT and its committees and panels; and the operation of the DCT library. In addition, the Chief, SIB, serves as a member of the Director of the Division of Cancer Treatment's OD staff, and the Director's Advisory Committee which formulates and carries out policy for the DCT and is composed of the Associate Directors of the Division and the Division's Chief Administrative Officer. The Branch Chief also participates as a member of the NCI's Institutional Review Board and participates in clinical research with the senior investigators of the Medicine Branch.

PUBLICATIONS

Cancer Treatment Reports (CTR), a primary source scientific journal dealing with preclinical and clinical cancer treatment, is in its twenty-fourth year of continuous publication. From 1959 to 1968 the journal, then known as Cancer Chemotherapy Reports, was issued 6-10 times a year depending on the number of manuscripts submitted and accepted for publication. Most, but not all, manuscripts were reviewed by outside referees as well as by members of the Editorial Board. In 1968 the journal expanded to three distinct parts:

- Part 1: Original research, both experimental and clinical;
- Part 2: Comprehensive, lengthy chemotherapy studies involving a great deal of tablular material; and
- Part 3: Program information including study protocols, clinical brochures, toxicology reports, and review articles.

In January 1976, the journal was renamed Cancer Treatment Reports (CTR). The three-part separately numbered system was dropped and the journal began monthly publication. The journal now considers unsolicited and previously unpublished manuscripts of original research under eight major categories:

1. Full length manuscripts
2. Brief Communications
3. Letters to the Editor
4. Clinical Trials Summaries
5. Special Features
6. Current Controversies in Cancer Management
7. Meeting reports
8. Mini-symposia

All material submitted for consideration in CTR is subject to review as deemed appropriate by two or more outside referees and a member of the Editorial Board. In addition, the journal publishes meeting announcements and program information for oncology programs throughout the world.

Submissions in 1982 and 1983

During 1982, the journal received 572 research manuscripts. During the first 6 months of 1983, 320 manuscripts have been submitted. CTR has continued to publish on a regular monthly schedule since the summer of 1981. The Branch is currently evaluating micro-computer/word processing systems that would upgrade and streamline office operations and facilitate more efficient processing, indexing, and tracking of manuscripts submitted for publication in CTR and Symposia. This project is being performed in anticipation of the fact that GPO is planning to convert its publishing operations to electronic transfer of printed material to its typesetting and printing contractors in the future. Therefore, the capacity to handle electronic transfer is an another consideration in the selection of a computer system.

Comparative data on the CTR series of publications are shown in Table 1 below.

TABLE 1

Year	Manuscripts Received	Manuscripts Accepted	Issues Published	Pages Published	Reviewers
1973	244	207	7	1100	167
1974	286	180	11	1823	213
1975	365	261	9	1888	295
1976	478	302	12	2021	402
1977	422	313	9	1771	432
1978	578	364	12	2168	557
1979	740	667	10	2175	695
1980	576	269	4	827	595
1981	499	212	6	1159	339
1982	572	282	12	2149	470
1983*	320*	297*	6*	609*	

* From January 1 to June 30, 1983

Sections of the Journal

Full Length Manuscripts - This section contains the results of clinical or preclinical research which has been given a high priority for publication as full length manuscripts ranging from 7 to 15 printed pages.

Brief Reports - This section contains short clinical manuscripts and abbreviated reports of preliminary research which can be processed more quickly than full length manuscripts. They are cost efficient in terms of editorial effort and journal space and serve to increase the number of research studies that can be disseminated to the readership. Submissions to this section continue to be excellent.

Special Features - The special feature section contains invited editorials and commentaries from authoritative scientists who are invited by the members of the Editorial Board to comment on subjects of current interest in cancer treatment. In 1983, the SIB invited prominent investigators to provide editorial commentaries on the following subjects:

1. Camitta, B., Kun, L., Glicklich, M., Oechler, H., Adair, S., and Pinkel, D.,: Doxorubicin-Vincristine Therapy for Wilms' Tumor: A Pilot Study. Cancer Treat Rep 66(10): 1791, October 1982.

   D'Angio, G.: Invited Comment on the Milwaukee Pilot Study of Doxorubicin-Vincristine, Therapy for Wilms' Tumor. Cancer Treat Rep 66(10): 1795, October 1982.

2. Sladek, N. E., Smith, P. C., Bratt, P. M., Low, J. E., Powers, J. F., Borch, R. F., and Coveney, J. R.: Influence of Diuretics on Urinary General Base Catalytic Activity and Cyclophosphamide-Induced Bladder Toxicity. Cancer Treat Rep 66(11): 1889, November 1982.

   Woolley, P. V.: Editorial on Management of Drug Toxicity: Cancer Treat Rep 66(11): 1901, November 1982.

3. Pizzo, P. A. and Schimpff, S. C.: Strategies for the Prevention of Infection in the Myelosuppressed or Immunosuppressed Cancer Patient. Cancer Treat Rep 67 (3): 223. March 1983.

Letters to the Editor - During the past several years, CTR has observed a steady increase in the number of Letters submitted and accepted for publication. Letters to the Editor are also reviewed prior to acceptance and respond to previously published articles or represent unique case reports.

Clinical Trials Summaries - This section was introduced in January of 1981 to conserve journal space while further increasing the number of reports that could be published each month. Clinical Trials Summaries are concise summaries of data from clinical trials that have produced negative results. Authors are limited to 300 words of text and are encouraged to summarize patient characteristics, as well as response, toxicity, and survival data in tabular form. This format, which is concise and can be processed and

published quickly and efficiently by the editorial staff, has permitted publication of results from an increased number of clinical trials that contain essential, albeit negative, clinical research. The response to this new format has been positive and an increasing number of authors are submitting manuscripts in this format. At the end of each year, the editorial staff prepares a table summarizing all of the information presented in the Clinical Trials Summaries published during the year.

Current Controversies in Cancer Management - This new section contains papers by prominent investigators who have been invited to address controversial aspects of cancer treatment. The following articles were published in this section:

1. Mayer, R. J., Weinstein, H. J., Coral, F. S., Rosenthal, D. S., and Frei, E.: The Role of Intensive Postinduction Chemotherapy in the Management of Patients with Acute Myelogenous Leukemia. Cancer Treat Rep 66(7): 1455, July 1982.

   Thomas, E. D., Clift, R. A., and Buckner, C. D.: Marrow Transplantation for Patients with Acute Nonlymphoblastic Leukemia Who Achieve a First Remission. Cancer Treat Rep 66(7): 1463, July 1982.

   Preisler, H. D.: Therapy for Patients with Acute Myelocytic Leukemia Who Enter Remission: Bone Marrow Transplantation or Chemotherapy? Cancer Treat Rep 66(7): 1467, July 1982.

2. Rosen, G. and Nirenberg, A.: Chemotherapy for Osteogenic Sarcoma: An Investigative Method, Not a Recipe. Cancer Treat Rep. 66(9): 1687, September 1982.

   Lange, B. and Levine, A. S.: Is It Ethical Not To Conduct a Prospectively Controlled Trial of Adjuvant Chemotherapy in Osteosarcoma. Cancer Treat Rep 66(9): 1699, September 1982.

3. Young, L. S.: The Role of Granulocyte Transfusions in Treating and Preventing Infection. Cancer Treat Rep 67(2): 109, February 1983.

   Schiffer, C. A.: Granulocyte Transfusion Therapy. Cancer Treat Rep 67(2): 113, February 1983.

4. Byhardt, R. W. and Cox, J. D.: Is Chest Radiotherapy Necessary in Any or All Patients with Small Cell Carcinoma of the Lung? Yes. Cancer Treat Rep 67(3): 209, March 1983.

   Cohen, M. H.: Is Thoracic Radiation Therapy Necessary for Patients with Limited-Stage Small Cell Lung Cancer? No. Cancer Treat Rep 67(3): 217, March 1983.

5. Cox, J. D., Komaki, R., and Byhardt, R. W.: Is Immediate Chest Radiotherapy Obligatory for Any or All Patients with Limited-Stage Non-Small Cell Carcinoma of the Lung? Yes. Cancer Treat Rep: 67(4): 327, April 1983.

Cohen, M. H.: Is Immediate Radiation Therapy Indicated for Patients with Unresectable Non-Small Cell Lung Cancer? No. Cancer Treat Rep 67(4): 333, April 1983.

Proceedings of Scientific Meetings:

During the past several years, CTR has been approached frequently to publish the proceedings of scientific meetings sponsored by the Division. In 1982 the following proceedings were published in CTR:

1. Symposium on Contemporary Issues in Hodgkin's Disease: Biology, Staging, and Treatment. Cancer Treat Rep 66(4): 601-1067, April 1982.

2. Workshop on Small Cell Anaplastic Carcinoma of the Lung (Ashford Castle, Ireland). Cancer Treat Rep 67(1): 1-43, January 1983.

Editorial Board

The Editorial Board includes the Editor-in-Chief and twelve associate editors. Dr. Robert E. Wittes, Associate Director, Cancer Therapy Evaluation Program, has assumed the post of Editor-in-Chief replacing Dr. John S. Macdonald. Each year three editors rotate off the board and three new members are added. Provisions have been made to allow editors to have an additional year on the board at the discretion of the Editor-in-Chief and the Director, DCT. An Advisory Editorial Board of 15 members has also been established to supplement the areas of expertise represented by the associate editors. New Advisory Board members will be appointed in 1984 for a two year period. The official policies of the journal are contained in the official charter which was established in 1975.

Coverage of CTR in Current Contents and Related Publications

Since 1967, CTR has been listed in Current Contents, Life Sciences. In 1973, CTR was included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England. The Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications also includes CTR. This additional coverage of material presented in the journal has increased the demand for subscriptions, particularly in other countries.

In 1964, CTR began sending copies of each issue to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973, CTR established a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infordata International in Chicago, which publishes in the Index to the Periodicals of the US Government. In 1980, CTR began to send advanced copies for abstracting and indexing purposes to the Franklin Institute in Philadelphia.

Office of Management and Budget Approval

In January of 1983, a request for a 3 year continuation of funds was submitted to the OMB. This document outlined the purpose and scope of CTR, the publication costs incurred by the staff, a breakdown of the distribution of each issue, including the categories of subscribers, and the justification for continuing the publication. The Department has approved our request and forwarded it to the Office of Management and Budget for continued approval of publication.

Distribution of CTR

The Scientific Information Branch is responsible for maintaining the courtesy mailing list for CTR. CTR is available without cost to 3225 qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office (GPO). The approximate distribution, which varies with each issue, is shown on Table 2 on the following page. In the past several years, the number of paid subscriptions has increased tremendously. The number will undoubtedly continue to rise because of the limits set for free subscriptions by the OMB each time the journal receives OMB clearance. The demand for specific back issues remains high; most requests are for the proceedings of meetings.

SYMPOSIA

The publication of Symposia as an intermittent supplement to CTR has been given general approval. This permits the Branch to publish the proceedings of important scientific conferences sponsored by the Division dealing with cancer treatment research. Each issue is approved on an individual basis. The ability to publish symposia permits the SIB to devote an entire issue to subjects of interest to the cancer research community.

The SIB is in the process of publishing three symposia which will be issued as supplements to CTR in Cancer Treatment Symposia in the fall of 1983 and early 1984 entitled:

1. Proceedings of the United States-Japan Meeting on Drug Development and Cancer Treatment Research.

2. Proceedings of the Workshop on Patterns of Failure After Cancer Treatment.

3. An Interdisciplinary Program for Radiation Oncology Research.

TABLE 2

Distribution

1. Official Use (distributed at no cost)

   a. Federal Government

      NIH employees 193

      NCI contractors and grantees 840

      FDA employees 17

      VA employees 41

      VA libraries and hospitals 79

      PHS employees 10

      Armed Services (employees and libraries) 24

   b. State agencies 9

   c. Research institutes (including libraries) 551

   d. Medical schools and universities (including libraries) ... 216

   e. Hospitals (including libraries) 384

   f. Special advisory groups 24

2. Free distribution

   a. Foreign investigators and institutions 390

   b. Foreign libraries 101

   c. Pharmaceutical and related industries 59

   d. Individuals (such as medical practitioners) 287

3. Superintendent of Documents

   a. Paid subscriptions..... 3400

   b. File copies 73

   c. Depository Libraries 465

   TOTAL 7163

PDQ

In October of 1982, the National Cancer Institute established a new database on cancer and cancer treatment which is known as PDQ (Physician/ Patient Data Query). This database is now available for use through the MEDLARS System of the National Library of Medicine at over 2000 organizations including most biomedical libraries and research centers in the United States. PDQ presently contains information on NCI-sponsored protocols from the Institute's CLINPROT file that have been reformatted into a "user cordial" language and format. The file contains the titles, objectives, and entry criteria of over 900 active protocols supported by the NCI throughout the country. Linked to each protocol in PDQ is the name and address of the protocol coordinator, and all affiliated investigators at participating institutions, and the name of a key contact person at each institution. This first version of PDQ represents the initial phase of a cancer information system that is being designed to assist both physicians and patients to obtain information about cancer treatments by interacting with a computer. A series of statements on state-of-the-art treatment for each cancer type is being developed by an Editorial Board composed of leading intramural and extramural scientists from the NCI. The PDQ system will enable individuals to query the system to find qualified physicians with recognized expertise in the management of specific cancers. When queried about specific cancers, PDQ will provide general information on appropriate approaches to treatment (surgery, chemotherapy, radiotherapy or combination of more than one modality) by stage and important histologic type. The target date for the activation of the interactive component of the PDQ system is July 1, 1983.

Over the last year, the Chief, SIB, has also been involved in the development of this new Institute-wide initiative. The development of this new database has involved program leaders from all NCI operating Divisions. As leader of one of the management teams (Team C) the Branch Chief has been charged with the responsibility of overseeing all of the data-gathering functions for the PDQ system. This has involved integrating the efforts of prominent scientists within each of the operating Divisions of the NCI, distinguished scientists in the extramural community, and an Editorial Board to gather all active treatment protocols, the names of cancer specialists, and to develop the patient-oriented and physician-oriented treatment information being mounted on "user friendly" computer software. This has entailed the responsibility of coordinating all functions of the PDQ Editorial Board as they relate to the development of these materials and the review of treatment protocols.

Literature Research Section

The Literature Research Section serves the Division of Cancer Treatment by providing information from published literature on all aspects of the therapy of cancer. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy, and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies.

Literatures Services - The Section received and filled more than 300 requests for information during the year. Responses were provided as comprehensive or selected bibliographies, computer print-outs, abstracts, and copies of articles. Approximately 200 of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline, and Cancerline. Clinical searches were done for such subjects as Phase I studies in children, development of second tumors following therapy, hyperpigmentation in patients undergoing chemotherapy, and drug or hormonal therapy of prostatic cancer. Bibliographies were prepared, or updated, for several compounds included in the Project to Review Older Drugs, various interferons and thymosins, and for drugs of current interest such as etoposide, diaziquone, mitoxantrone, fludarabine phosphate, hexamethylenebisacetamide, deoxyspergualin, and caracemide. Searches were also done for the mechanism of action of anthracyclines, asparaginase rescue of antifolates, NAD analogs, and antineoplastic copper compounds. A total of 19 bibliographies were prepared for compounds discussed at 6 Decision Network meetings.

The Section co-ordinates NCI access to the on-line databases of the Medlars automated bibliographic search system. In this role, the Section serves all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. These computer services aid in the searching and facilitate the answering of requests. Response to over 80 requests were in the form of computer print-outs only. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for members of the staff on specific subjects of continuing interest.

A further responsibility of the Section is the maintenance of the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 70 journals are regularly received incluing many of the cancer journals, abstracting and indexing secondary sources, and chemical, biomedical or information secience journals of special interest to the Section and other personnel in the building.

During the past year, the Section continued the responsibility for extracting data and coding extramural protocols for input into the Cancer Therapy Evaluation Program Information System until this assignment was given to the contractor. An average of 44 protocols were handled per month.

Collaboration with Clinical Oncology Program

Other major accomplishments in 1982-1983 include participation of the Chief, SIB, in the clinical therapeutic trials performed by the Medicine Branch, COP, DCT, NCI. General accomplishments and publications are summarized under the report entitled Clinical Trials and Miscellaneous Clinical Investigations (Project Report 201-CM-03403-16M).

Other Publications Include:

1. Hubbard, SM: Cancer treatment research: The nurse's role in clinical trials of cancer therapy. In Woods, ME and Kowalski, J. (Eds.). Nursing Clinics of North America 17(4): 763-783, 1982.

2. Hubbard, SM and Jenkins, J: Part I: An overview of current concepts in the management of patients with testicular tumors of germ cell origin. Nursing '82 6(1): 39-47, 1983.

3. Hubbard, SM and Jenkins, J: Part II: An overview of current concepts in the management of patients with testicular tumors of germ cell origin. Nursing '82 6(2): 125-139, 1983.

4. Elder, DE, Greene, MH, and Fraser, MC: Dysplastic Nevus Syndrome Workshop. Hubbard, SM, Chairperson. Educational Symposium and Educational Workshop Booklet. Proc. ASCO, 5/22-24/83, San Diego, CA pp. 80-89.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1982 - September 30, 1983

I. Introduction

The Developmental Therapeutics Program (DTP) has primary operational responsibility for all aspects of the preclinical development of antitumor drugs for the Division of Cancer Treatment (DCT). The extramural component of the DTP is located in the Blair Building in Silver Spring, Maryland, where directed drug development activities are contract-supported and research in biochemistry and pharmacology is administered through the grant mechanism. The DTP intramural laboratory operation conducts anticancer drug and other pre-clinical cancer treatment-related research in Building 37 on the NIH campus in Bethesda.

The extramural program, which is devoted to the acquisition, antitumor evaluation, formulation, large-scale drug production and toxicology studies on new candidate anticancer drugs, is managed by seven Branches: Drug Synthesis and Chemistry, Natural Products, Drug Evaluation, Animal Genetics and Production, Pharmaceutical Resources, Toxicology, and Information Technology. An eighth Branch, Extramural Research and Resources, is responsible for the management of cancer-related chemistry, biochemistry and pharmacology grants.

The intramural program is conducted through five Laboratories: Medicinal Chemistry and Biology, Molecular Pharmacology, Tumor Cell Biology, Chemical Pharmacology, and Experimental Therapeutics and Metabolism. Intramural research is supportive of both new drug studies and basic investigations in cancer-related biochemical processes and molecular biology.

The Office of the Associate Director is responsible for the leadership and management of the DTP and the accomplishment of the goals and objectives of the DCT pre-clinical program. The progress of potential clinical candidates through the Decision Network process is summarized in Table I.

Seven drugs completed toxicology during 1982 (Table I, Decision Network 3). All of these, except Taxol, had INDA's filed during that year and are presently in Phase I clinical trial. The Taxol formulation problem discovered just prior to INDA filing will be resolved soon. Eight new compounds achieved Decision Network 2A status during the one year period covered in Table I. Six compounds were approved to begin toxicology (Decision Network 2B).

DCT has committed resources to obtain the information required on several compounds recommended by the committee directing the Project to Review Older Drugs (PROD) effort. Compounds will continue to be added to this category.

II. Accomplishments

A. Extramural Program

1. Acquisition of new materials as potential anticancer drugs

a. Drug Synthesis and Chemistry Branch (DS&CB)

The fundamental responsibility of the DS&CB is the acquisition and management of the flow of unique synthetic compounds for evaluation as potential anticancer agents. The acquisition activities resulted in the following: (a) 19,700 chemical structures were acquired and processed through the preselection process; (b) 10,300 compounds were acquired and assigned NSC numbers; (c) 316 new suppliers were added; (d) 604 compounds showed confirmed activity against P388 leukemia in vivo and (e) five synthetic compounds achieved Decision Network 2A status.

Computer methods for preselecting compounds for large-scale screening against P388 based on molecular fragment structure-activity statistics (Hodes model) have been in operation for the past four years. This year a model for therapeutic index was added. Work was begun on estimates for activity with regard to partition coefficients. In addition, the P388 leukemia estimates are being augmented with estimates of activity in L1210 leukemia and B16 melanoma.

DS&CB has worked closely with the Radiation Research Program (RRP) and the Radiosensitizer/Radioprotector Working Group by monitoring the two radiosensitizer contracts and identifying and acquiring compounds for both the radiosensitizer and radioprotector screening contracts. A non-nitro compound has emerged as a promising radiosensitizer and is undergoing detailed evaluation. In addition, NSC 301467 (SRI-2508) has entered Phase I clinical trials in the U.S. and NSC 347503 (RSU-1069) is undergoing preclinical toxicology testing in England.

The DS&CB plays a key role in the identification of compounds and renders other support services in connection with the new NCI initiative to develop clinical compounds through EORTC. Examples of compounds under collaborative development include NSC 224070, 127716, 267702, 340847 and 345842.

b. Natural Products Branch (NPB)

Natural product research has a long history of producing novel and unusual types of chemical structures which show many types of biological activity. The NPB has actively pursued acquisition, isolation, structure determination, and testing of compounds from microbial, plant and animal sources in order to obtain new leads for further development in the NCI anti-cancer drug program.

This year four fermentation contractors evaluated 13,232 new cultures. Thirteen percent (1,744) of these fermentation broths were active in one or more of the pre-screens. Forty percent (693) of the in vitro actives have been regrown and tested in vivo against the P388 lymphocytic leukemia model. Twenty-two percent (152) were found active. We obtained 27 pure antibiotic materials from our contractors and 56 from outside sources. A total of 289 fermentation derived materials are currently in the NCI tumor panel.

The PRI-FCRF fermentation contract is primarily a pilot plant research and development contract where kilogram quantities of toyocamycin have been produced as an intermediate for the tricyclic nucleoside, tricribrine phosphate, which is currently in clinical trials. The optimization, scale-up and the purification of largomycin is being developed to prepare sufficient quantities for schedule dependency studies, formulation and toxicology. Scale-up studies to produce Rapamycin, which is a possible candidate for clinical study are underway. The structure of the novel compound, Fredericamycin, was completely elucidated. Stability studies of this compound were undertaken in preparation for formulation, schedule dependency studies and toxicological studies.

During calendar year 1982, the NPB worldwide literature surveillance program initiated the acquisition of 232 new pure natural products of which 179 (77%) were donated and the remaining 53 compounds (23%) were from contractors. The compounds acquired were approximately evenly distributed between domestic (51%) and foreign (49%) sources and came from a variety of sources with commercial organizations supplying 23%, universities 57% and research institutes and government laboratories supplying the remaining 21%.

2. Biological Evaluation

a. Animal Genetics and Production Branch (AG&PB)

The primary function of the AG&PB is the production of healthy laboratory animals with properly defined genetic characteristics. The AG&PB is also responsible for the proper functioning of the Tumor Respository.

The AG&PB adjusted to the changing DTP needs (e.g., tumor panel changes). The changing needs of other users including new strain requirements (primarily FCRF intramural) have been handled expeditiously.

A program was instigated to study the feasibility/cost efficiency of upgrading conventional laboratory facilities to a pathogen exclusion status. The efficacy of using an aerosol delivered Sendai vaccine for strain sensitive rodents was established.

A pilot study regarding the practicability of freezing fertilized mouse embryos has progressed to the extent that strains that are currently not in usage, but where future demand is a probability, may be "stored" in this fashion.

b. Drug Evaluation Branch (DEB)

DEB objectives are the conduct of tasks essential to the pre-clinical development of new drugs to clinical trial, and the development of improved models for drug discovery and evaluation.

From April 1, 1982 - March 31, 1983, 11,104 new materials (9,482 synthetic agents and 1,266 natural products) were screened for the first time.

The pre-clinical aspects of the 1976-1983 eight-tumor panel experiment were completed. The results and recommendations for future modifications of the panel were presented to the DCT Board of Scientific Counselors and implemented in June 1982. The new plan retains the P388 *in vivo* prescreen. Currently, agents active in the pre-screen are tested in a panel consisting of L1210, B16, the mammary xenograft and one new model, mouse tumor M5076. A major feature of the new screen is an additional step in which active drugs are tested (1) against two other tumors from the old panel (CD and C8), (2) against drug resistant tumor variants, and (3) in other assays designed to increase the challenge for the compound.

Analysis of the 1976-1982 panel experiment reinforced the value of P388 as an *in vitro* pre-screen. Nevertheless, exclusive use of this lone assay in the process of new antitumor drug discovery may limit the potential for uncovering agents with unique mechanisms of action. DEB is exploring cost-efficient models which may show chemosensitivities qualitatively different from P388 as potential ancillary pre-screens.

In testing 79 compounds that failed the P388 *in vivo* pre-screen, 14, including several novel structures, were found active in the human tumor clonogenic assay.

Route and schedule dependency studies were completed for Tiazofurin, Didemnin B (NSC 325319), and Bactobolin (NSC 325014). Pre-clinical activity of experimental clinical formulations was validated for Caracemide, Isopropyl pyrrolizine (NSC 278214), Ara-5-Ac (NSC 281272), Bisbenzimidazole (NSC 322921), Didemnin B, and Trimetrexate glucuronate (NSC 352122).

The DEB organized a symposium (February 1983) on Cellular Resistance to Anticancer Drugs. Two areas were identified as deserving priority for future research: (1) reversal of resistance to natural products by calcium blockers or other agents and (2) identification and characterization of human tumor cells showing pleiotropic drug resistance.

DTP has initiated the implementation of the National Cooperative Drug Discovery Groups (NCDDG) program. It is anticipated that each NCDDG will be multi-disciplinary and multi-institutional. The goal is to apply strong scientific rationale to the design and synthesis of new anticancer therapies and to the selection or development of the most informative pre-clinical assays for recommending new agents. A pre-RFA announcement elicited about 300 expressions of interest. We plan to have the RFA published in July 1983, to receive applications in October 1983, and to make awards in the Spring of 1984. The NCDDG program will be conducted concommitantly with the current drug screening and evaluation program. They are envisioned as mutually complementary.

3. Formulation and bulk chemical procurement

The goal of the Pharmaceutical Resources Branch (PRB) is to provide high quality chemicals and pharmaceutical products to the Programs of the DCT in a timely and efficient manner. The achievement of this goal involves coordination of chemical, analytical and pharmaceutical activities.

During this reporting period, the chemical prep lab contractors prepared 136 compounds. Twenty-three drugs, totaling 159 kilograms, were delivered for pharmaceutical product use. These drugs included Tiazofurin (NSC 286193); Dihydro-5-azacytidine (NSC 264880); Ara-AC (NSC 281272); Methyl G (NSC 32946); ICRF-187 (NSC 169780); and CBDCA (NSC 241240).

Thirteen compounds were prepared by the radiosynthesis contractors including N-Methylformamide (NSC 3151); CBDCA (NSC 241240); Ara-AC (NSC 281272); and Tiazofurin (NSC 286193).

The analytical activities of the Branch have experienced significant changes due to increased demands from the Food and Drug Administration for additional information on low level impurities, validation of analytical systems and preparation reference standards. The analytical contract effort was increased last year from a 14 staff-year to an 18 staff-year effort.

During the last reporting period, several difficult formulation projects were successfully completed. Most notable was Spiromustine (NSC 172112) which is a highly water insoluble and unstable compound with interesting antitumor activity. Other dosage forms developed recently include Menogarol (NSC 269148); Caracemide (NSC 253272); and Fludarabine Phosphate (NSC 312887).

Over 500,000 injectable units and 1,000,000 oral dosages were manufactured for NCI-sponsored clinical trials by PRB contractors during this reporting period.

4. Pre-clinical toxicology

The primary function of the Toxicology Branch (TB) is the toxicologic characterization of potential antineoplastic agents prior to Phase I clinical trials. The Branch achieves its objectives in pre-clinical toxicity and safety evaluation through the operation and management of a prime toxicology contract in which the qualitative and quantitative toxicological profiles of antitumor drugs and modalities are determined in animals.

Toxicology reports for individual INDA submissions were completed on Tiazofurin, Acodazole, Caracemide, Taxol, Menogarol, and Rapamycin. A cooperative effort was established with the Blood Level Working Group whereby toxicity data will be developed in conjunction with pharmacokinetic studies to provide as complete a "biological profile" as possible on each new drug. These data will be correlated with those obtained from Phase I clinical studies for their predictive value in Phase I dose escalation schemes.

New protocols in which F-344 rats replaced CDF_1 mice in the optional rodent toxicity tests were submitted to the FDA to update the Master Agreement. Three drugs (Bisbenzimidazole, Didemnin B, and Bactobolin) are currently under test using the rat as well as the dog to ascertain drug induced toxicities. This protocol modification resulted from review of toxicity data derived from the mouse. Several problems noted in this species stemmed from the small size of the animal. The mouse is now used solely to establish initial clinical doses. Currently, informal guidelines for long-term toxicity testing of antineoplastics are being developed and evaluated.

5. Information technology

The Information Technology Branch (ITB) has been working on the installation of the new Drug Information System (DIS). The goal of the DIS is to provide DCT staff with online access to all preclinical drug development data via an interactive computer system. This is expected to reduce dramatically the amount of paper used by the current DTP operation, facilitate usage of data, and increase the timeliness of responses to queries.

Work on this task is progressing rapidly and five of the six major DIS modules are now in various stages of implementation. The sixth, with which DCT orders samples of chemicals from its inventory to be tested, is already in operation. This has allowed the program to curtail the contract which previously had monitored this function. As new DIS capabilities become available, cost savings are beginning to appear in a variety of areas, such as the chemical database contract. Meanwhile, the amount of searching being conducted by DCT staff, particularly the chemists and biologists, is increasing as searching becomes less dependent on ITB intervention and thus more convenient. Most of the files in the DIS are designed to be automatically updatable as often as daily. Thus, currency is maximized with little staff effort.

The ITB collaborates actively with other programs within DCT and elsewhere. In this connection, the Branch has developed toxicity databases which are used by various other groups and it has also produced a variety of technical reports on subjects of interest to DCT scientists. In collaboration with Lederle Laboratories, a structure-activity relationship study of anthracyclines has been completed and, together with the NCI's Division of Cancer Cause and Prevention, ITB has made the database of Chemical Carcinogens, many of which are of interest to DCT as cytotoxic agents, generally available in a search system. In addition, an effort is being made to produce and disseminate a publicly accessible version of the NCI antitumor testing database without infringing upon the numerous confidentiality agreements that exist between NCI and suppliers of chemicals.

6. Grants in pharmacology and biochemistry

The primary function of the Extramural Research and Resources Branch (ERRB) is to administer extramural research supported by grants for pre-clinical development of antineoplastic drugs. The major areas of emphasis in the Biochemistry and Pharmacology research program are: drug design and synthesis, natural products development, experimental therapeutics, selective screening for activity, comparative pharmacology and toxicology and mechanism of drug action.

During Fiscal Year 1983, the Branch supported 314 research projects totalling 34.9 million dollars. The distribution of projects among the major categories reflects efforts to ensure a balanced research program in accordance with the need and current priorities for drug development.

BIOCHEMISTRY AND PHARMACOLOGY PROGRAM
BY SUB-CATEGORY
FY 1983

	Number of Grants	Total Amount (Thousands)
SYNTHESIS & CHEMISTRY	121	$ 11,917
NATURAL PRODUCTS	30	2,324
SCREENING & EXPERIMENTAL THERAPEUTICS	28	2,884
COMPARATIVE PHARMACOLOGY	19	1,850
OTHER PRECLINICAL ASPECTS	9	978
MECHANISM OF ACTION	101	9,948
PROGRAM PROJECTS	6	5,014
TOTAL	314	$ 34,915

B. Intramural Program

1. Laboratory of Chemical Pharmacology (LCP)

LCP studies are chiefly concerned with elucidating the pharmacological properties of new anticancer agents, and include an evaluation of their disposition and metabolism, their mechanism of action, the mechanisms by which tumor cells become resistant to them, and their potential adverse effects.

A major thrust of research within the Laboratory is directed toward understanding the mechanism(s) of selective toxicity of antitumor agents. Research from this Laboratory has shown that the isolated perfused rat liver exports uridine at concentrations similar to that found in plasma. Furthermore, plasma appears to be an important source of performed pyrimidines that can be utilized by cells with an intact salvage pathway and thus could be an important factor determining the effectiveness of certain antimetabolite antitumor agents. It was found that when cultured L1210 cells were incubated in various constant concentrations of uridine, the concentrations of uridine equivalent to that found in the plasma substantially inhibited de novo pyrimidine biosynthesis. The salvage pathway was less sensitive than the de novo pathway to intracellular uracil nucleotide concentrations indicating a preferential use of salvage over de novo synthesis.

Techniques to monitor the flux through the de novo pyrimidine pathway in tumors and host tissues in vivo were refined and extended. This method quantitates the incorporation of pathway precursors (labeled with stable isotopes of carbon and nitrogen) into tissue uracil nucleotide pools by GC/MS. It allows, for the first time, an evaluation of differential effects of inhibitors of de novo synthesis in normal vs. tumor tissues in vivo. PALA, a known inhibitor of de novo synthesis, was used as a test compound

and $^{13}CO_2$ was used as the labeled precursor. Pathway flux was inhibited for at least 48 hours in Lewis lung carcinoma, a PALA-sensitive tumor line; whereas, in L1210, a PALA resistant tumor line, recovery of pathway activity occurred at 12-24 hours. This recovery of flux did not correlate with the recovery of aspartate transcarbamylase activity. A search for inhibitors of uridine salvage yielded two highly active compounds. One compound is a newly synthesized nitropyrimidine. Resolution of the optical isomers of this nitropyrimidine demonstrated that the salvage-inhibitory properties reside in only one of the isomers. This compound is the first uridine derivative to block uridine phosphorylation in intact cells. Studies are in progress with this compound to evaluate possible therapeutic enhancement of inhibitors of de novo pyrimidine biosynthesis. The other active compound is tiazofurin (NSC 286193), an inhibitor of purine biosynthesis. Both compounds were found to block the utilization of uridine by cultured L1210 cells.

Studies of the hepatic regulation of circulating purines were continued. Purine bases and nucleosides were quantitated by HPLC analysis and metabolic interconversions of added purines were determined by radiotracer methodology. Hypoxanthine and inosine were rapidly metabolized to uric acid and approximately 2% of each of these tracers was salvaged by the liver and incorporated into liver purines. Adenosine was rapidly deaminated and the resulting inosine metabolized to uric acid. Adenosine was salvaged to a greater extent by the liver with 20% of the total radioactivity being incorporated into liver purines. Adenine was found to be exported by the liver in increasing concentrations until it reached 1μ M and then plateaued. No adenosine was found in the effluent of the perfused liver, even when the liver was exposed to a 10μ M concentration of hypoxanthine. Thus, it appears that the liver exports adenine and not adenosine as had been previously postulated. Studies were continued on the in vitro and in vivo cytotoxicity of 2'-deoxy-5-azacytidine (DAC). In vitro cytotoxicity studies with DAC indicate a maximum cell kill of 3 to 4 logs is achievable with optimal conditions of concentrations and exposure time. In vivo cytotoxicity studies with DAC indicate log cell kills of greater than 7 logs at optimal doses. Reasons for this discrepancy are being studied. Advanced L1210 tumor in mice with late treatment, indicate drug resistant cells (deoxycytidine kinase deficient mutants) may be a major problem with DAC. Collaterally active drugs drugs such as cytoxan, BCNU, dihydro-5-azacytidine, 3-deazauridine tiazofurin are being studied as potential solutions to this drug resistance. Biochemical modulation of DAC activity was found to occur with thymidine and immune modulation occurred with pyran copolymer.

A basic understanding of the movements of drugs and physiological materials between blood, brain, CSF, and systemic tissues is essential for an appreciation of normal CNS and systemic functions as well as the changes in these transport systems caused by various CNS and systemic malignancies. Improved techniques for measuring and analyzing transport phenomena have been developed. Among these

developments are: (1) better methods of determining blood-brain and blood-tissue transfer constants for materials of low to moderate permeability; (2) a quantitative and more relevant assessment of drug delivery and uptake by normal and tumorous tissues in terms of tissue extraction fractions and microvascular permeability-surface area products; and (3) double-label quantitative autoradiographic and co-imaging techniques to facilitate regional tissue analysis and correlation. Intra-arterial drug infusions achieved increased exposure of the target organ and decreased exposure of other tissues when a drug was rapidly metabolized, blood flow through the infused artery is slow, and infused drug is rapidly broken-down in the target organ. For most small brain tumors, blood flow and transcapillary influx (the two major components of intravascular drug delivery) were similar to that of normal brain; however, for large brain tumors, blood flow was generally reduced but transcapillary influx ranged from normal to greatly increased.

2. Laboratory of Experimental Therapeutics and Metabolism (LETM)

The general goal of the LETM is to generate basic information which will lead to new insights into molecular toxicology, tumor biology, biochemistry and pharmacology that will contribute to the improvement of cancer treatment.

Techniques for isolating and characterizing the major cell-types of lungs from a number of animal species have been developed. From rabbit lung, both alveolar type II and bronchiolar Clara cells have been isolated. These cell-types are presently being used to quantify the distribution of the pulmonary cytochrome P-450 system using acetylaminofluorene as substrate.

Chemically-reduced paraquat was found to form at least two distinct products in the presence of NADPH or NADH. This reaction was sensitive to the presence of oxygen. In both rat liver and lung subcellular fractions, no interaction between paraquat and the pyridine dinucleotides was apparent under either aerobic or anaerobic conditions.

Doxorubicin was concentrated in isolated dog lung during a 50 minute perfusion in situ. The uptake of drug increased with time, but was saturable at perfusate concentrations exceeding 20 nmol/ml. Changes in the kinetic model parameters suggested that, at high perfusate concentrations, doxorubicin influx was inhibited. These studies have lead to a Phase I clinical trial utilizing the hemiperfusion technique in metastatic sarcoma patients. The surgical and perfusion procedures were successfully performed in all the patients examined to date. However, in human lung, doxorubicin accumulation was considerably slower than in the dogs. The concentration of drug in biopsied tumors from the patients was consistently less than the surrounding tissue.

A cerium precipitation method is being developed to investigate localized production of superoxide in lung. Extensive studies using Ce/H_2O_2 in the presence of a range of antioxidant enzymes and superoxide traps were undertaken in order to characterize the interaction of cerium with reduced oxygen. The rate of this reaction was biphasic, pH-dependent and inhibited by ascorbic acid, superoxide dismutase and albumin but not by ethanol or mannitol.

A reliable model of MeCCNU renal damage in BDF mice and Fischer 344 rats has been developed. Preliminary studies show the Fischer rat to provide a relevant model also for studying the nephrotoxicity of other nitrosoureas. Streptozotocin was found to be acutely nephrotoxic, as measured by in vivo renal function tests, whereas, low doses of chlorozotocin result in a chronic progressive nephropathy.

Single doses of 8 mg BCNU/kg or 80 mg BCNU/kg inhibited lung glutathione (GSSG) reductase by 11% and 82%, respectively. The inhibition was very persistent, lasting up to seven days after a single dose of BCNU. The multi-dosing regimen of 5 mg BCNU/kg/week for six weeks results in the development of severe pulmonary damage. After three weeks of BCNU dosing, GSSG reductase activity decreased to 50% of control values and GSSG levels rose to 227% of control values. The glutathione redox system is part of an important anti-oxidant defense mechanism in pulmonary cells and further studies are under way to explore whether impairment of this system by BCNU ultimately leads to or contributes to pulmonary toxicity.

Hydroxy-fatty acids were released from phospholipids by enzymatic hydrolysis. The acids were then separated by an HPLC procedure using a reverse-phase chromatography system and were detected and quantitated, against synthesized standards, by UV absorption at 235 nm. Livers and lungs from rats given carbon tetrachloride, or from mice given paraquat, showed HPLC peaks indicative of hydroxy-fatty acids, although none corresponded exactly with the synthetic standards available. Further studies will continue to probe the development and potential applicability of this methodology to the investigation of the role of lipid peroxidation in tissue injury.

The LETM has investigated the effects on several cellular immune functions of the transfer of nude genes to beige mice. The resulting viable, double homozygous recessive bg/bg nu/nu (beige-nude) mice combine the relative absence of T-cell function in regular nude mice, known to have high NK levels with the very low NK activity of beige mice. Therefore, such double immune deficient mice provide new possibilities for studying immune surveillance, particularly for establishing to what extent NK cells take part in host defense against spontaneous, induced, or transplantable tumors. No spontaneous malignant tumors have been observed in these beige-nude mice thus far.

3. Laboratory of Medicinal Chemistry and Biology (LMCB)

The LMCB was established in order to provide a facility capable of antitumor drug discovery from the stage of drug design and synthesis through biochemical and pharmacological characterization to Phase I clinical trial.

Within the Laboratory, work has continued during the past year both on the synthesis of new drugs which will be clinically useful in the treatment of cancer, and/or the development of analytical support for pharmacokinetic studies of agents which originated within the Section, and which are now in Phase I/II clinical trial (AZQ; Dihydro-5-azacytidine (DHAC); Spirohydantoin mustard).

Several avenues are being pursued in the design and synthesis of new agents. A dinucleotide (TAD), the active metabolite of the Phase I agent, tiazofurin (NSC 286193), was successfully synthesized. This anabolite acts by inhibiting the enzyme IMP dehydrogenase, a key step in the purine biosynthetic pathway. Over the past year, six analogues of TAD have been synthesized, and the biological activity of three of these has been evaluated. Of particular interest is the analogue adenine-D-ribose-phosphate-phosphate-D-ribose-selenazole-4-carboxamide, a compound several-fold more active than TAD as an IMP-dehydrogenase inhibitor.

Biochemical studies have continued on the mode of action of tiazofurin. In studies of a spectrum of six murine and six human tumor cell lines, with varying degrees of sensitivity to the drug, it was established that an excellent correlation exists between the ability of a tumor line to convert tiazofurin to its active anabolite TAD, and the cytotoxic effects of the drug. In related studies, the factors responsible for the greater antitumor activity of the corresponding selenium analogue (selenazofurin) as compared to the parent sulfur compound (tiazofurin) have been explored. It has been established that selenazofurin, like tiazofurin, undergoes anabolism to an analogue of NAD, and that the latter is also a potent inhibitor of IMP dehydrogenase.

The development of new analytical techniques for agents of interest to the NCI has continued. The objectives of these studies are to establish the structure and purity of new antitumor agents, and also to elucidate reaction mechanisms and to develop assays for the quantitation of these drugs in physiological samples. Mass spectrometry, gas liquid chromatography NMR and HPLC techniques are used. In collaboration with the Clinical Oncology Program, extensive clinical pharmacology studies have been continued with LMCB-developed agents now in Phase I/II clinical trial (AZQ and DHAC), and the major human pharmacokinetic parameters for these two compounds have been elucidated. Extensive studies also have been carried out with the differentiation-inducing agent HMBA (hexamethylene bis-acetamide). Experiments have been undertaken in rats to determine the feasibility of maintaining HMBA concentrations *in vivo* which match exposure conditions *in vitro*.

A rapid and simple gas chromatographic method incorporating an internal standard and involving the direct analysis of plasma or urine has been developed for the measurement of HMBA in these fluids. A limit of detection of less than 50 μg/ml is obtained and the small amount of plasma required for sample workup permits multiple sampling from treated animals. A single in bolus dose of 800 mg/kg HMBA results in plasma levels well above 5 mM. This compound is rapidly eliminated from the plasma [$t_{1/2}(\alpha)$ = 5.5 min; $t_{1/2}(\beta)$ = 122 min] with a total body clearance of 7 ml/min/kg.

At the molecular level, studies have been initiated on the 5-azacytidine analogue, arabinosyl-5-azacytosine (araAC; NSC 281272), a compound recently synthesized within the LMCB. AraAC has murine antitumor activity comparable to that of 5-azacytidine and to arabinosylcytosine (araC); possibly more important, however, is its activity in vivo against all three human tumor xenografts (mammary, colon and lung) as compared to 5-AC (inactive), 2'-deoxy-5-AC (inactive) and araC (active against the mammary tumor only).

A definitive study of the renal handling of cis Pt has now been completed. The renal clearance of free cisplatin was the same as the glomerular filtration rate (GFR). Probenecid resulted in a 70% increase in GFR, but N-methylnicotinamide did not affect GFR. Cisplatin is excreted by a process which includes active reabsorption via the organic acid transport system. Platinum excretion was correlated with urinary pH and not urinary volume. A therapeutic advantage may be gained by regional IA administration of cisPt for pelvic neoplasma. These studies, together with ancillary pharmacokinetic studies, are now being extended to the platinum analog, CBDCA, presently in early clinical trial.

Studies have continued on antineoplastic agents which act on tubulin, a protein critical for cell division. Based on earlier studies from this laboratory which demonstrated conditions in which tubulin-dependent GTP hydrolysis was totally dependent on taxol (NSC 125973), we were able to develop an assay for the drug sensitive to 0.1 μM. Conditions were established suitable for measuring serum concentrations, and a preliminary pharmacokinetic study has been performed in rabbits. Although taxol appears to be protein-bound in serum, it is rapidly cleared with α-phase and β-phase half-lives of 2.7 and 42 min, respectively. This assay is suitable for human pharmacokinetic studies, and has been made available to clinical research groups which will be conducting Phase I trials with this agent.

Efforts have also continued to elucidate the mode(s) of resistance to the clinically important antitumor agent, melphalan (L-PAM). Murine L1210 leukemia cells resistant to L-PAM have been sensitized in vitro and in vivo by reducing the cellular concentration of the tripeptide, glutathione. Pharmacological reduction of tumor cell glutathione can be achieved by constant infusion, via osmotic pumps, of D,L-buthionine-S, R-sulfoximine, an inhibitor of glutathione biosynthesis, into tumor-bearing mice. The therapeutic implications

of these observations in L-PAM sensitive and resistant human ovarian tumor cell lines are now being explored in collaboration with the Medicine Branch, COP.

4. Laboratory of Molecular Pharmacology (LMP)

The Laboratory of Molecular Pharmacology is engaged in studies to determine the molecular mechanisms of action of anticancer drugs. Much of the work concerns bifunctional alkylating agents, DNA intercalating agents, and related drugs. The major questions are what DNA lesions or other effects on DNA, are produced by these drugs; to what extent are specific DNA lesions repaired in various cells; and how do the effects on DNA relate to cytotoxicity.

The progressive development of the alkaline elution technique reached the point a few years ago where it became possible to measure DNA interstrand crosslinks (ISCs) and DNA-protein cross-links (DPCs) without major interference between these two types of bifunctional lesions. This lead to the discovery that human tumor cell strains generally fall into two groups which differ in the formation of chloroethylnitrosourea (CNU)-induced ISCs, even when the formation of DPCs is similar. The strains that generated high frequencies of CNU-induced ISCs, had been found to be deficient in DNA guanine-O^6-methyl transferase activity, a phenotype that is designated Mer^-. The Mer^- strains had an increased sensitivity to CNU-induced cell killing, thus indicating that the ISCs, or associated lesions, are important in the killing of human tumor cells. We had proposed the hypothesis that, among the DNA alkylation products produced by CNUs, there are guanine-O^6 chloro-ethylations which can slowly react with the opposite DNA strand to form ISCs. Our hypothesis further was that these DNA-chloroethyl monoadducts are rapidly removed by the guanine-O^6-alkyl transferase activity in Mer^+ tumor cells, as well as in normal human cells, thereby preventing ISC formation and enhancing cell survival.

During the past year, the hypothesis was further tested by the use of DNA methylating agents which inactivate the guanine-O^6-alkyl transferase. It was found that pretreatment of Mer^+ tumor cells or normal human cells with methylating agents increased CNU-induced ISC formation from undetectable to easily measurable levels. The results were thus in accord with our hypothesized mechanism and supported the importance of ISC formation in the killing of human tumor cells by CNUs.

The effects of AZQ on DNA in cells and subcellular systems were studied, and new insights into its mechanism of action were obtained. The chemical structure of the drug suggests that it could have two types of reactivity: (1) oxidation-reduction activity which could lead to free-radical reactions, and (2) bifunctional alkylation activity which could generate crosslinks. In accordance with this expectation, AZQ was found to generate (a) DNA strand breaks in an NADPH-requiring and superoxide dismutase-inhibitable reaction and (b) DNA interstrand crosslinks (ISCs) by means of an alkylation

reaction which was greatly enhanced by reduction of the AZQ quinone. The enhanced alkylating and enhanced ISC formation by reduced AZQ, however, was unexpected. These findings suggest that, contrary to the generally accepted view, AZQ may be a bio-reductive alkylating agent and might be useful in the treatment of hypoxic tumor tissue.

In previous work, the LMP had tested the then prevalent idea that the intercalator-induced DNA strand breaks were induced by a free radical mechanism. Free radical scavangers, thiourea and dimethylsulfoxide were studied. It was found that, although thiourea reduced the frequency of protein-associated strand breaks brought on by an intercalator treatment, dimethylsulfoxide had the opposite effect. The increase in intercalator-induced strand breakage by dimethylsulfoxide was striking and unexpected. Although a good hypothesis to explain this phenomenon is still not unavailable, the possibility that it might be related to the induction of terminal differentiation by DMSO was studied. A structure-activity analysis, however, disclosed that other differentiation inducers of the polar-planar type that were tested failed to increase intercalator-induced strand breakage, and in fact tended to surpress it. The stimulation by dimethylsulfoxide is thus exceptional and remains unexplained.

Previously, the LMP discovered and characterized two new histone variant proteins which were named H2A.X and H2A.Z. It was found that, in the absence of DNA synthesis, histones continue to be synthesized, although at a much slower rate than in S-phase. Certain of the variant proteins, however, showed strikingly different relative synthesis rates compared to the S-phase pattern, and different patterns were observed in quiescent (G_0) cells as opposed to G_1 or G_2-phase cells. During the past year, studies were carried out on the functional nature of the histone synthesis that continues in the absence of DNA synthesis. Histones synthesized during G_1 and G_0 were found to become incorporated stably into nucleosomes. Preliminary studies, however, indicated that there may be some histone turnover in G_0 cells. The stimulation of DNA repair synthesis by UV did not increase the synthesis of G_0 or S-phase histone variants. Inhibition of DNA synthesis by hydroxyurea, or other S-phase specific inhibitors, suppressed the synthesis of basal histone variants (e.g., H2A.X and H2A.Z) to a much lesser degree than the inhibition of S-phase histone synthesis. Studies of a variety of DNA synthesis inhibitors is in progress in order to determine whether the remaining basal histone synthesis pattern is always the same.

5. Laboratory of Tumor Cell Biology (LTCB)

The objectives of the LTCB are to develop, implement, and analyze data obtained from studies of cellular proliferation, cell differentiation, and biochemical growth characteristics of normal and malignant mammalian cells both in vivo and in vitro. Particular attention is given to hematopoietic cells, their normal behavior and especially changes seen during leukemogenesis. Because of

unusual access to human blood cells and because of the interest of this group, there is special focus on human leukemias and lymphomas, and acquired immune deficiency syndrome (AIDS). It is anticipated that an enhanced understanding of cell regulatory mechanisms will permit the optimal use of antitumor agents in the therapy of cancer and the development of new approaches.

During the past year, significant progress has been made in the investigation of AIDS, human T-cell leukemia virus (HTLV), human T-cell growth factor (TCGF), onc genes and cellular differentiating agents.

Twenty new neoplastic human T-cell lines have been developed from patients with T cell malignancies and AIDS. Seroepidemiological studies show that HTLV antibodies are present in some patients with AIDS.

Seroepidemiology studies show that HTLV is widespread in certain areas of Japan, West Indies, South East U.S.A., China, Russia, Africa, Malaysia, and Central and South America. HTLV has been transmitted into cord blood lymphocytes, bone marrow and adult peripheral blood T-cells. The amino acid sequence analysis of HTLV p24 and p15 has been completed. There are some similarities in the amino acid sequence of HTLV p24 and BLV p24 and HTLV p15 and BLV p12. Cloning of HTLV-I and HTLV-II genomes and flanking cellular sequences from cells of several T-cell leukemia patients has been accomplished. A molecular epidemiology survey for HTLV in human tumors has been carried out and shows the presence of HTLV provirus in a number of tumor tissues. Nucleotide sequencing of HTLV-I LTR has been completed. Cloning of a gene, HT-3, that is specifically transcribed in HTLV infected T-cells has been accomplished.

A partial amino acid sequence of purified TCGF has been determined. Cloning of the gene for TCGF has been accomplished.

Cloning and chromosomal mapping of four human cellular onc genes has been completed. Cloning of genes from ALL and AML cells that transform mouse fibroblast cells in vitro has been carried out.

A differentiation inducing activity (DIA) has been shown to act synergistically with retinoic acid in inducing differentiation of fresh cells from patients with acute promyelocytic leukemia. Retinoic acid was shown to induce the synthesis of NAD^+-glycohydrolase (NADase) in HL-60 cells. Other inducers of differentiation such as dimethylsulfoxide, hypoxanthine, 1,25-dihydroxyvitamin D_3, and butyric acid do not induce this enzyme. The induction of NADase has been adapted as a very sensitive and rapid assay for retinoids that induce differentiation of HL-60.

TABLE I

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/82 - 3/31/83)

NSC Number	Name	Compound Type*
	Decision Network 2A	
156492D	Discreet	S
303812D	Discreet	SS
308847	1H-Benz[de]isoquinoline-1,3(2H)-dione, 5-amino-2-[2-(dimethylamino)ethyl]-; Benzisoquinolinedione	S
329680D	Discreet	S
339638	2H-Pyran-2-one, 5,6-dihydro-6-[3,6,13-trihydroxy-3-methyl-4-(phosphonooxy)-1,7,9,11-tridecatetraenyl]-, monosodium salt; Pyranone Phosphate	NP
347512	4H-Benzopyran-8-acetic acid, 4-oxo-2-phenyl-; Flavone Acetic Acid	S
353451	Imidazo[5,1-d]-1,2,3,5-tetrazine-8-carboxamide, 3-(2-chloroethyl)-3,4-dihydro-4-oxo-; Azolastone	S
356894	Heptanamide, 7-[(aminoiminomethyl)amino]-N-[2-[[4-[(3-aminopropyl)amino]butyl] amino]-1-hydroxy-2-oxoethyl]-, (+)-; Deoxyspergualin	NP
	Decision Network 2B	
278214	Carbamic acid, (1-methylethyl)-, [5-(3,4-dichlorophenyl)-2,3-dihydro-1H-pyrrolizine-6,7-diyl]bis(methylene) ester; Isopropyl Pyrrolizine	S
284356	4,8-Ethenopyrrolo[3',4':3,4]cyclobut[1,2-f]isoindole-1,3,5,7(2H,6H)-tetrone, octahydro-; Mitindomide	S
322921	Phenol, 4-[5-(4-methyl-1-piperazinyl)[2,5'-bi-1H-benzimidazol]-2'-yl]-, trihydrochloride; Bisbenzimidazole	S
325014	Propanamide, 2-amino-N-[3-(dichloromethyl)-3,4,4a,5,6,7-hexahydro-5,6,8-trihydroxy-3-methyl-1-oxo-1H-2-benzopyran-4-yl]-, [3S-[3,4α(R*), 4aβ,5β,6α]]-; Bactobolin	NP

NSC Number	Name	Compound Type*
325319	Didemnin B	NP
352122	D-Glucuronic acid, compd. with 5-methyl-6-[[(3,4,5-trimethoxyphenyl)amino]methyl]-2,4-quinazolinediamine (1:1); Trimetrexate	S
	Decision Network 3	
125973	Benzenepropanoic acid, β-(benzoylamino)- -hydroxy-, 6,12b-bis(acetyloxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl-5-oxo-7,11-methano-1H-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester, [2aR-[2aα,4β,4aβ,6β,9α(R*,βS*),11α,12α,12aα,12bα]]-; Taxol	NP
172112	1,3-Diazaspirol[4,5]decane-2,4-dione, 3-[2-[bis(2-chloroethyl)amino)ethyl]-; Spirohydantoin Mustard; Spiromustine	S
253272	Acetamide, N-[(methylamino)carbonyl]-N-[[(methylamino)-carbonyl]oxy]-; Caracemide	S
286193	2-β-D-Ribofuranosyl-4-thiazolecarboxamide; Tiazofurin	S
301467	1H-Imidazole-1-acetamide, N-(2-hydroxyethyl)-2-nitro-; SR 2508	S
305884	Acetamide, N-methyl-N-[4-[(7-methyl-1H-imidazo[4,5-f]quinolin-9-yl)amino]phenyl]-, monohydrochloride; Acodazole HCL	S
312887	9H-Purin-6-amine, 2-fluoro-9-(5,O-phosphono-β-D-arabinofuranosyl)-; Fludarabine phosphate	S
	Decision Network 4	
141633	Cephalotaxine, 4-methyl-2-hydroxy-2-(4-hydroxy-4-methylpentyl)butanedioate ester; Homoharringtonine	NP
241240	Platinum, diamine[1,1-cyclobutanedicarboxylato(2-)-O,O']-, (SP-4-2)-; Carboplatin; CBDCA	S
256927	Platinum, dichlorodihydroxybis(2-propanamine)-, (OC-6-33)-; CHIP	S

NSC Number	Name	Compound Type*
	Decision Network Special	
324360	Benzamide, N,3,4-trihydroxy-; Hydroxymic Acid derivative	S
	Decision Network PROD	
177248	1,2,4-Triazin-3(4H)-one, 4-β-D-ribofuranosyl-, 1-oxide; Uricytin	NP
364989	1-Bis-(β-chloroethyl)amino-2-aminoethane	S
45410	Pyrazole	S
57155	N,N"-bis[p-(N'-methylamidino)phenyl]-terephthalamidine; Tetrahydrochloride	S

*S = synthetic
NP = natural product
SS = semi-synthetic (natural product modified synthetically)

ANNUAL REPORT OF THE DRUG SYNTHESIS & CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 - September 30, 1983

The fundamental responsibility of the Drug Synthesis and Chemistry Branch (DS&CB) is the acquisition and management of the flow of unique synthetic compounds for evaluation as potential anticancer agents in the Developmental Therapeutics Program (DTP).

The DS&CB achieves its central mission by engaging in a variety of Program activities, namely, selective acquisitions, preselection model development, task order syntheses, contract syntheses, storage and distribution and computer assisted structure-activity analysis. A new project, "synthesis of congeners and prodrugs", has been initiated. The computerized structure-activity model has been refined to include therapeutic index estimates. In addition, DS&CB supports the Radiotherapy Development Program (RDP) and the European Organization for Research on Treatment of Cancer (EORTC) Program.

The contracts managed by DS&CB are outlined in Table I. Presently, the DS&CB is staffed with six professionals and three technical and clerical personnel.

Acquisitions

The function of the Acquisition Section is to ensure the continuous flow of approximately 10,000 compounds, selected from a large pool of available compounds for the primary screen. To fulfill this objective, the Section is engaged in the following activities: (1) developing and maintaining extensive contacts with industries and academic scientists for acquiring compounds with unique structural features and biological activity; (2) maintaining an effective selection ratio of inputs of compounds; (3) developing and implementing selection criteria; (4) developing computer models to facilitate selective acquisition; (5) acquiring compounds for the primary screen and Tumor Panel evaluations; (6) conducting structure-activity correlation studies; and (7) monitoring the discreet agreements with industrial suppliers. Specifically, the acquisition activities resulted in the following: (a) 19,700 chemical structures were acquired and processed through the preselection "guantlet"; (b) 10,300 compounds were acquired and assigned NSC numbers; (c) 316 new suppliers were added and (d) 604 compounds showed confirmed activity against P388 leukemia; (e) five synthetic compounds namely: NSC's 308847, D156492, D336628, D201047 and D329680 passed DN2A.

Preselection Model

Computer methods for preselecting compounds for large-scale screening against P388 based on molecular fragment structure-activity statistics (Hodes model) have been in operation for the past four years. This year

a model for therapeutic index was added. Work was begun on estimates for activity with regard to partition coefficients. In addition, the P388 leukemia estimates are being augmented with estimates of activity in L1210-leukemia and B16 melanoma.

Structure-Activity Analysis

Detailed structure-activity analyses based on our large chemical-biological data base are an essential part of our acquisition and synthesis activities. Such large-scale analyses of our data files have beome feasible because of the chemistry-biology interlink. Examples of structure-activity analyses include: purines and pyrimidines and quinones.

Resynthesis - Task Order Contracts

The functional responsibility for this project rests with the Drug Synthesis Section. The task order mechanism continues to be a cost-effective way to resynthesize compounds of program interest that are not available in sufficient quantities from the original investigators. The Task Order mechanism makes available 8 master contractors who have the expertise to synthesize a wide variety of organic and inorganic compounds. For this reporting period, we have awarded 37 individual projects consisting of 267 compounds. Compounds selected for synthesis include PS actives, toxics, LE actives, radiosensitizers/radioprotectors, special requests from intramural scientists, panel compounds, rationally designed compounds and bioactive compounds emerging from literature surveillance. During this reporting period, we received 239 compounds synthesized through this mechanism. Presently, the master contracts are being recompeted.

Storage and Distribution

The objective of this project is the storage, distribution, inventory and documentation of synthetic materials, crystalline natural products and bulk clinical drugs. During the past year, Flow Laboratories, Inc., the storage and distribution contractor, has shipped more than 1,200 compounds per month to contract screening laboratories, formulation laboratories, NCI and NIH researchers, and independent investigators in 40 states of the U. S. and 30 foreign countries. The vast majority of compounds are shipped within 48 hours after receipt of the request. The contractor is continuing the physical inventory of compounds (more than 400,000 containers) held in the DS&CB repository starting with DN, Panel and SAC compounds. The contractor continues to shelve and inventory compounds returned by the screening laboratories and shelve reference samples. The contractor recently completed the task of replacing the wooden drawer chests with steel shelving and the transfer of compounds to the new units. The contractor interacts closely with the DS&CB, DEB, NPB, PRB and ITB, as well as the acquisitions contractor and the chemical information contractor.

Literature Searches

The chemical search component of the DS&CB plays an integral part in supporting the search needs of the various Program elements of the Branch, namely, the acquisition of new novel synthetics, contract synthesis and grants. It also provides the scientific community with information on the compounds

screened in the Program. During this period, 1,900 full structure and 500 substructure searches were performed. In addition, the Questel/Darc and Dialog data base systems were utilized to access synthesis methods for selected compounds.

The literature surveillance program reviews the massive number of compounds published each month, estimated at 30,000 per month, and prepares a list of approximately 1,000 of the most interesting ones for review by DS&CB. These compounds are scored by the Hodes model and reviewed by chemists. The compounds that are finally selected are acquired either through mail requests or task order synthesis.

Radiotherapy Program Support

DS&CB works in close cooperation with Dr. Pistenmaa and his staff and the Radiosensitizer/Radioprotector Working Group. We actively support the Radiotherapy Development Program in several ways, namely; (a) monitoring the two radiosensitizer contracts, and (b) identifying and acquiring compounds for both the radiosensitizer and radioprotector screening contracts. A non-nitro compound has emerged as a promising radiosensitizer and is undergoing detailed evaluation. NSC 301467 (SRI-2508) has entered Phase I clinical trials and NSC 347503 (RSU-1069) is undergoing preclinical toxicology testing in England.

EORTC-Compounds in Development

The DS&CB plays a key role in the identification of compounds and renders other support services in connection with the new NCI initiative to develop clinical compounds through EORTC. Examples of compounds under development include NSC 224070, 127716, 267702, 340847 and 345842.

Synthesis of Congeners and Prodrugs

The objectives of this new project are to design and synthesize (a) congeners of viable leads which require either better activity or wider spectrum of activity to reach DN_2 level; (b) prodrugs of leads that offer greater stability and/or improved solubility. This approach of "optimization is already showing promise, for example, 1) soluble derivatives, NSC's D361429, 361813, 364188 of NSC 284356 have been synthesized and these have shown P388 activity equivalent to the parent; 2) a stable congener of NSC 159159, aza analog NSC D361456 has been synthesized. NSC D361456 shows better activity than the parent (DN_2 level).

TABLE 1

CONTRACTS - FY 1983

Contractor	Investigator	Contract No.
Dynamac Corporation	Gray	NO1-CM-07332
Flow Laboratories	Dorian	NO1-CM-27505
Maxima Corporation	Sobers	NO1-CM-17400
SRI International	Lee	NO1-CM-17485
Starks C. P., Inc.	Schultz	NO1-CM-87206
Alabama, Univ. of	Baker	NO1-CM-07355
Illinois Institute of Technology Research Institute	Uchic	NO1-CM-07359
Research Triangle Institute	Seltzman	NO1-CM-07352
SISA, Inc.	Razdan	NO1-CM-07354
Southern Research Institute	Montgomery	NO1-CM-07260
Southwest Foundation for Research and Education	Rao	NO1-CM-07356
SRI International	Acton	NO1-CM-07351
Starks Associates, Inc.	Starks	NO1-CM-07357
Alabama, University of	Baker	NO1-CM-27571
Georgia Technological Research Institute	Zalkow	NO1-CM-27517
The Research Foundation of the State University of New York at Buffalo	Anderson	NO1-CM-27570

PUBLICATIONS by STAFF

1. Lomax, N. R. and Narayanan, V. L.: Chemical Structures of Interest to the Division of Cancer Treatment - Compounds in Development - Drugs With Clinical Activity: Vol. III, 1983.

2. Naff, M. B., Plowman, J. and Narayanan, V. L.: Anthracyclines in the National Cancer Institute Program. In El Khadem, H. S. (Ed.) Anthracycline Antibiotics. Academic Press, N. Y., 1-57, 1982.

3. Narayanan, V. L. Strategy for the Discovery and Development of Novel Anticancer Agents. In Reinhoudt, D. N., Connors, T. A., Pinedo, H. M. and van de Poll, K. W. (Eds.): Structure-Activity Relationships of Antitumour Agents. Martinus Nijhoff Publishers, The Hague, 1983.

4. Narayanan, V. L. and Lee, W. W. Development of Radiosensitizers - A Medicinal Chemistry Perspective. In Schnitzer, R. J. (Ed.): Advances in Pharmacology and Chemotherapy: 19, 155-205, 1982.

5. Narayanan, V. L., Paull, K. D., and Nasr, M. Computer Assisted Structure-Activity Correlations: α- β- Unsaturated Ketones, Lactones, Lactams and Related Michael Type Acceptors as Antitumor Agents. Advances in Pharmacology and Chemotherapy. In Press

6. Pettit, G. R., Paull, K. D., Herald, C. L., Herald, D. L. and Riden, J. R. The Structure of the Benzene-Maleimide Photosynthetic Product (Mitindomide). Can. J. Chem. In Press

PUBLICATIONS BY CONTRACTORS

Ten publications were received from the contractors.

SEMINARS:

The following seminars were sponsored by DS&CB:

1. "Pro Drugs of Benzimidazole Carbamates - Synthesis and SAR"
   Dr. Roger D. Haugwitz, Squibbb Institute for Medical Research

2. "The Development, Anticancer Activity and Mechanism of Action of Azolastone (NSC 353451)"
   Dr. John A. Hickman, The University of Aston, Birmingham, England

3. "Twenty Year's Progress in Synthetic Anticancer Agents"
   Dr. Yun Feng Ren, Shanghai Institute of Materia Medica, Academia Sinica, Shanghai, China

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07101-08 DSCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Computer Methods for Drug Preselection Based on Structure-Activity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Dr. Louis Hodes, Acquisition Section, DS&CB, DTP, DCT, NCI, NIH

COOPERATING UNITS *(if any)*

Chemical Abstracts Service

LAB/BRANCH

Drug Synthesis & Chemistry Branch

SECTION

Acquisition Section

INSTITUTE AND LOCATION

NCI, NIH, Silver Spring, Maryland 20910

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Structure-Activity relationships have been adapted to aid in selecting compounds for large-scale screening in mouse lymphocytic leukemia (P388). Molecular fragment statistics from compounds tested in P388 are used to estimate antitumor activity and novelty. These estimates are examined by a medicinal chemist along with the structures of over 30,000 potential acquisitions per year to decide which compounds to screen. Results from P388 on about 30,000 compounds that have been through this system indicate that the estimates are useful. This year a model for therapeutic index was installed and work was begun on estimates for activity with regard to physical parameters. A more comprehensive model includes data from L1210 leukemia and B16 melanoma. In addition, these methods have yielded an automated literature surveillance project.

ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 - September 30, 1983

One of the major objectives of the Division of Cancer Treatment is to discover novel types of compounds with antineoplastic activity which can provide a basis for new chemotherapeutic agents.

In this regard, natural product research has a long history of producing novel and unusual types of chemical structures which show many types of biological activity, and indeed the basic structural types of various classes of natural products have led to development of many major classes of chemotherapeutic compounds. The investigation of natural substances for anticancer activity provides new types of compounds for evaluation which possess unusual properties, and can lead to new drug classes for chemotherapeutic testing in cancer treatment.

The Natural Products Branch has actively pursued acquisition, isolation, structure determination, and testing of compounds from microbial, plant and animal sources in order to obtain new leads for further development in the NCI program.

The major program areas of the Natural Products Branch are: 1) contract research directed toward isolation of new agents from active extracts from microbial sources; 2) world-wide literature surveillance for the acquisition of natural products with demonstrated biological activity or novel structural types for evaluation; 3) procurement and preparation of large quantities of active agents for drug formulation, tumor panel testing, toxicology and clinical studies.

Objectives

The objectives of the Natural Products Branch are: (a) to acquire a wide of unique chemical compounds of natural origin through donations and contracts for evaluation as potential antitumor agents; (b) to conduct a world-wide program of literature surveillance to identify materials of interest for active acquisition; (c) to collaborate with suppliers of compounds to develop new derivatives and analogs of compounds of interest; (d) to procure or produce additional quentities of compounds under study to assure a sufficient supply of material for detailed biological evaluation and subsequent developmental studies including pharmaceutics and tocicology.

Organization and Staffing

The Branch is organized into three segments, the Office of the Chief, the Fermentation Section and the Plant and Animal Products Section. Many tasks require interaction between these segments and the Branch personnel are assigned duties in whichever of the areas require their expertise depending on changing program needs. The present full time staff consists of four professionals and two secretaries. The contracts managed by the Branch are outlined in Table 1.

Table 1.

Natural Products Branch Contracts

Contractor	Investigator	Contract No.	Program Area
Bristol Myers	Bradner	NO1-CM3-7556	Fermentation
PRI-FCRF Fermentation Program	Flickinger	NO1-CM2-3910	Fermentation Scale-up
Microbial Chem. Res. Foundation	Umezawa	NO1-CM5-7009	Fermentation
Polysciences Inc.	Boettner	NO1-CM3-7557	Plant Products Scale-up
Warner-Lambert	French	NO1-CM3-7614	Fermentation
Upjohn Company	Neil	NO1-CMO-7380*	Fermentation
University of Illinois	Loub	NO1-CM3-7553	Literature surveillance

*Expired 12/31/82

Fermentation Section

The objective is the isolation and development of novel antineoplastic drugs derived from microbial fermentations. The section maintains five contracts in support of these tasks. Four contracts are devoted to the isolation of unique organisms and to systematically evaluate the microbial world for its

ability to produce novel anticancer agents. The work includes selection and screening of microbes, fermentation, fermentation development, genetic and culture research, chemical isolation, identification and scale-up production of active materials of interest for NCI evaluation.

The program is using novel and/or improved techniques and unusual substrates to obtain unique organisms in the hope of generating numerous new structural leads. In vitro prescreens are also constantly improved or changed to increase the efficiency of the program in selecting potential leads and decrease the in vivo testing load for primary fermentations. Currently used screens include enzyme inhibition, tubulin binding, microbial inhibition, phage induction, DNA binding, antimetabolite activity and cytotoxicity against various cell lines.

Active fermentations in the prescreens are tested in P388 leukemia in vivo and when confirmed active are assigned to the chemist for isolation, purification and identification of the active component for NCI evaluation. Further work on the compound is based on its activity, novelty of structure or superiority to the parent compound in the case of an analog. The antineoplastic agents are supplied by the four contractors. One other contract is maintained to support production of active agents in quantities sufficient for toxicology and clinical trials. This year the prescreens allowed the Fermentation contractors to evaluate 13,232 new cultures. One thousand seven hundred forty four (13%) of these fermentation broths were active in one or more of the prescreens. Six hundred ninety three (40%) of the in vitro actives have been regrown and tested in vivo against the P388 lymphocytic leukemia, of which one hundred fifty two (22%) were found active.

This year, we obtained 27 pure antibiotic materials from our contractors and 56 from outside sources. A total of 289 fermentation derived materials are currently in the NCI tumor panel.

The PRI-FCRF fermentation contract is primarily a pilot plant research and development contract where kilogram quantities of toyocamycin have been produced as an intermediate for the tricyclic nucleoside, tricribrine phosphate, which is currently in clinical trials. The optimization, scale-up and the purification of largomycin is being developed to prepare sufficient quantities for schedule dependency studies, formulation and toxicology. Gilvocarcin has been produced in gram quantities for evaluation in the entire tumor panel. Seveal pigments produced by the luteoskyrin-producing organism have been produced and are undergoing evaluation by the NCI. Preparation of several other leads (PR1350, PR1360, Anticapsin and Luteoskyrin) is in progress. Scale-up studies to produce Rapamycin, which is a possible candidate for clinical study are underway. The structure of the novel compound, Fredericamycin, has been completely elucidated. Stability studies of this compound were undertaken in preparation for formulation, schedule dependency studies and toxicological studies.

Plant and Animal Products Section

The acquisition of new active antineoplastic agents from higher plants and from animal products (marine, primarily) is the major function of this area. This is carried out through liaison with NIH grantees and other collaborating scientists and includes providing data from the extensive NCI files on extracts of plants and animals tested, providing in vivo screening services to establish priorities for leads to be fractionated and in vitro bioassay services to aid in isolation of active compounds. Pure compounds isolated are evaluated in NCI screens and when additional quantities are required these are procured by purchase from the investigator or are produced by the contract which is maintained for scale-up isolations. This contract also produces large quantities of plant derived drugs as needed for toxicology and clinical trials including taxol, indicine N-oxide and phyllanthoside.

Worldwide Surveillance of Natural Products

This function is primarily carried out in the Office of the Chief. The objective is to acquire new natural products with biological activities which may relate to anticancer effects, and compounds of a wide variety of new or unusual structural types which are worthy of screening for antitumor activity. The approach to identification of compounds for acquisition is multifaceted, and includes a contract for literature surveillance which identifies new natural product structures and biological activities, literature review by Branch Staff, personal contacts with scientists in universities, research intitutes and chemical and pharmaceutical companies, attendance at scientific meetings where new compounds are reported, and review of progress reports of NIH grantees. During calendar year 1982 a total of 232 new pure natural products were acquired of which 179 (77%) were donated and the remaining 53 compounds (23% were from contractors. The compounds acquired were approximately evenly distributed between domestic (51%) and foreign (49%) sources and came from a variety of sources with commercial organizations supplying 23%, universities 57% and research institutes and government laboratories supplying the remaining 21%. The distribution of these compounds by natural source is shown in Table 2.

Table 2

Pure Compounds Acquired Calendar Year 1982

	Contract	Donated	Total
Fermentation	25	56	81
Plant	16	119	135
Animal	12	4	16
Total	53	179	232

NSC#	Drug	Origin	Status
226080	Rapamycin	fermentation	Toxicology
125973	Taxol	plant	Toxicology
269148	Menogarol	fermentation	Toxicology
330500	Macbecin II	fermentation	Formulation
325319	Didemnin B	marine animal	Toxicology
328426	Phyllanthoside	plant	Formulation
325014	Bactobolin	fermentation	Toxicology
333856	Tetrocarcin A	fermentation	Analytical development
237020	Largomycin	fermentation	Bulk procurement
356894	Deoxyspergualin *	fermentation	Formulation
303812D	Discreet *	fermentation	Analytical development
339638	Pyranone phosphate deriv. *	fermentation	Formulation

*Passed Decision Network 2A FY 83

Contractor Publications and Patents

During this year contractors published 26 papers or abstracts which involved NCI support. Also six patents were issued to these contractors and four patent filings were submitted.

Staff Publication

Gillin, R., Reiner, D.S. and Suffness, M. Bruceantin, a potent amebicide from a plant, Brucea antidysenterica. Antimicrob. Agents Chemother. 22:342-345, 1982.

ANNUAL REPORT OF THE ANIMAL GENETICS & PRODUCTION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

The primary function of the Animal Genetics and Production Branch (AG&PB), Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators as follows: (1) DTP Screening programs and tumor panel; (2) other NCI research contracts (DCCP, DCBD, etc.); (3) NIH and FCRF Intramural Program: (4) Research Grants; (5) NIEHS, and (6) Veterans Administration Research facilities (surplus animals which are offered when available).

The AG&PB is responsible for the proper functioning of the Tumor Repository which includes:

1. Scheduled distribution of human/animal tumors to DTP contract screening and associated laboratories.

2. Distribution of tumors to qualified cancer research investigators upon request.

3. Monitoring tumor lines maintained by the Repository and contract screening laboratories for pathogenic contaminants.

4. Performing pilot studies with metastatic human/animal tumors to evaluate their potential for DTP Program usage.

The AG&PB assists the Toxicology Branch by assuring the production and distribution of adequate numbers of beagles which meet specified standards for their program.

The AG&PB is responsible for staffing and maintaining the DCT intramural Barrier Facility in Building 37.

The objectives of the AG&PB are to:

1. Continue to provide laboratory animals of the quality (from both an animal health and genetic integrity viewpoint) and quantity to meet the needs of the various programs using these services.

2. To upgrade experimental tumor monitoring services and provide necessary manipulations to assure that those tumors of importance to NCI Research Programs:

   A. Are free of pathogenic contamination

B. Meet individual tumor performance standards, e.g., growth rate, life span, metastatic potential, etc.

C. Meet individual tumor characterization requirements including the usage of histology, karyotyping, isoenzyme electrophoretic studies, etc. as needed.

3. To provide metastatic human/murine tumor models which offer potential for usage in DTP tumor panel and other DCT cancer research programs.

4. To maintain the Building 37 barrier facility in a fashion that will:

   A. Meet investigator requirements

   B. Exclude pathogenic contaminants

   C. Meet all accrediation requirements

5. To provide assurance that the FCRF Animal Production Facility continues to provide laboratory animals of superior quality and remains cost effective.

Accomplishments:

1. The AG&PB has adjusted to changing needs of DTP programs (e.g., tumor panel changes) and to unexpected delays in completing the phasing-out of several tumor panel models to the extent that screening laboratories have accomplished requested testing in a timely fashion.

2. Changing needs of other users including new strain requirements (primarily FCRF intramural) have been handled expeditiously.

3. A program has been instigated to study the feasibility/cost efficiency of upgrading conventional laboratory facilities to a pathogen exclusion status.

4. The efficacy of using an aerosol delivered Sendai vaccine for strain sensitive rodents, e.g., DBA/2 mice has been established.

5. Experimental tumor lines for DTP programs have been delivered as scheduled by DEB/AG&PB staff.

6. A pilot study regarding the practicability of freezing fertilized mouse embryos has progressed to the extent that strains that are currently not in usage, e.g., RFM/Un, but where future demand is a probability, may be alternatively "stored" in this fashion.

ANIMAL GENETICS & PRODUCTION BRANCH PROGRAM FUNDING

FY 1983

PRIMARY GENETIC CENTERS (4)	$5,041,000
Supply breeding nucleus for the animal program and athymic mice for drug evaluation.	
RODENT PRODUCTION CENTERS (4)	1,317,000
Large-scale production of inbred mice under both conventional and barrier controlled environment.	
HYBRID MOUSE PRODUCTION CENTERS (6)	827,000
Supply hybrid mice for the screening program.	
DIAGNOSTIC & HISTOCOMPATIBILITY PROJECTS (9)	1,036,000
To monitor animal health and genetic integrity.	
DEVELOPMENT OF STANDARDS & GUIDELINES (1)	30,000
For animal care and breeding.	
MAINTENANCE OF FROZEN TUMOR BANK (1)	229,000
FREDERICK CANCER RESEARCH FACILITY	1,623,000
CENTRALIZED REDERIVATION	98,000
Rederiving new starts from the NIH Repository into associated flora status.	
TOTAL	$10,201,000
Less Reimbursements (Including Grantee Collection)	2,820,000

ANNUAL REPORT OF THE DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

Drug Evaluation Branch (DEB) objectives are discovery of new anticancer agents, conduct of pre-clinical tasks essential or complementary to development of new drugs to clinical trial, and development of improved methods for drug discovery and evaluation. Principal products are recommendations of new drugs to the DCT Decision Network (DN) Committee, efficacy reports for inclusion in Clinical Brochures and Investigational New Drug Applications (INDA's), and communication of findings to clinical groups and the scientific community orally and via publication. As of April 30, 1983 Program was implemented through 15 contracts funded at over $6,000,000 (Table 1).

The 1976-1983 tumor panel experiment was completed. Results and recommendations for modifying the screen were presented to the DCT Board of Scientific Counselors and implemented in June 1982. The current plan (Figure 1) retains the P388 in vivo pre-screen. The 1976-1982 panel included five mouse tumors: leukemia L1210, melanoma B16, CD8F1 mammary tumor (CD), colon 38 (C8), and Lewis lung carcinoma (LL) and three human tumor xenografts: mammary (MX-1), colon (CX-1), and lung (LX-1). Currently (Figure 1), agents active in the pre-screen are tested in a smaller panel consisting of L1210, B16, and MX-1 - 95% of agents that met DN criteria against at least one tumor in the old panel were active against at least one of these three - and one addition, mouse tumor M5076. While new agents are presented to the DN Committee on the basis of panel activity, a major feature of the current screen is a third step in which active drugs are tested against two tumors from the old panel, CD and C8, against drug resistant tumor variants, and in other assays designed to increase the challenge for the compound.

Pre-screen

From April 1, 1982 - March 31, 1983 11,104 new materials (9,482 synthetic agents and 1,266 natural products) were screened for the first time.

Analysis of the 1976-1982 panel experiment reinforced the value of P388 as an in vivo pre-screen. Nevertheless, exclusive use of this lone assay in the process of new antitumor drug discovery may limit the potential for uncovering agents with unique mechanisms of action. DEB is exploring cost efficient models which may show chemosensitivities qualitatively different from P388 as potential ancillary pre-screens.

In Vivo Screening

The 1976-1982 tumor panel experiment was designed to maintain a steady flow of new agents for development to the clinic and to provide information relative to the following questions: (1) would the panel select new drugs that would be missed using L1210 alone; (2) do human tumor xenografts select the same or different drugs; and (3) do human tumor xenografts and mouse tumors derived from the same tissue select the same drugs?

From January 1977 through December 1983, INDA's were filed or were anticipated for 33 drugs for which panel results were instrumental in the decision to go forward. Fourteen of the 33 were not active against L1210. An additional 25 panel active drugs, 13 of which were not active against L1210, were in earlier stages of development to the clinic. Of 225 panel active drugs, 13 failed against L1210, B16, or MX-1 and each of the 13 was active against only one of the five remaining tumors in the old panel. The INDA was filed for one, Homoharringtonine (NSC-141633, HHT), active against C8 only. A Flavone derivative, NSC-347312, also active against C8 only, is in an earlier stage of development. Of 26 compounds active against one or more of the three human tumor xenografts, 22 were active against MX-1. INDA's were filed for N-Methylformamide (NSC-3051, NMF), active against all three xenografts and for Pentamethylmelamine (NSC-118742, PMM), active against MX-1 only. The Institute of Cancer Research (ICR), England (CM4-3736) had found PMM active against a non-panel human lung xenograft. INDA filing for Caracemide (NSC-253272), active against MX-1 only, is expected this year.

Addition of M5076 to the current (Figure 1) in vivo panel, stemmed from preliminary evidence that it is a "high yield" tumor capable of responding to compounds which are inactive against other current in vivo assays. M5076 exhibited specific patterns of organ metastasis (Southern Research Institute, SoRI, CM9-7309 and FCRF), was inhibited by macrophage activating agents (FCRF), and can be readily manipulated to determine drug availability to target cells under various tumor site and treatment route relationships (Arthur D. Little, Inc., ADL, CMD-7302). The Screening Operations Section (SOS) - responsible primarily for monitoring contractor conduct of in vivo screens (Table 1, Section 1), tracking of active agents through panel testing, and documentation of actions of the DCT Operating Committee (Table 1, Section 6) - also is responsible for the quality control of screening operations (Table 1, Section 5) and the establishment of standard protocols for in vivo screening. SOS evaluated the operational characteristics of the M5076 model under various experimental conditions and reviewed results of contractor tasks related to establishment of M5076 screening protocols.

SOS completed an update of in vivo screening protocols. Each updated protocol was submitted to the Investigational Drug Branch (IDB), CTEP for filing with the FDA. Publication of complete updated protocols is expected within the year.

The Selected Agents Section (SAS) reviewed pre-screen results, selected compounds for tumor panel testing, designed non-routine secondary screening experiments, conducted biochemical and detailed biological evaluation of active drugs, and prepared reports of pre-clinical therapeutic efficacy for presentation to DCT committees and for inclusion in INDA's and clinical brochures.

By April 1983, testing against all eight tumors had been completed for over 95% of the compounds assigned to the old panel. From April 1, 1982 - March 31, 1983 data for 674 P388 confirmed actives were reviewed. Since June 1982, 580 compounds had been assigned to the current tumor panel (Figure 1) and testing had been completed for 41%, 52%, and 33% against B16, L1210, and MX-1, respectively.

Table 2 shows, for the period April 1, 1982 - March 31, 1983, the number of compounds that passed major decision points toward acceptance as candidates for development to the clinic. Pre-clinical efficacy reports were submitted to IDB for five drugs: Taxol (NSC-125973), Spiromustine (NSC-172112), Caracemide,

Acodazole HCL (NSC-305884), and Fludarabine phosphate (NSC-312887). The report for Tiazofurin (NSC-286193) was updated.

Human Tumor Colony Forming Assays (HTCFA)

The Cell Culture Section (CCS) reported results from four contractors (Table 1, Section 2) showing that six tumor types: breast, colorectal, kidney, lung, melanoma, and ovary, were the more suitable for screening in vitro against fresh surgical explants. CCS developed a working protocol for screening in the HTCFA. In testing 79 compounds that failed the P388 in vivo pre-screen, 14, including several novel structures, were found active in the HTCFA. Simpler in vitro tests against serially transferrable human tumor cell lines may be suitable for large scale pre-screening.

In Vitro/In Vivo Correlations

Failure of the panel to provide information on correlative drug responses among human and mouse tumors of similar tissue origin was attributed to the limitation imposed by using just one human tumor and one mouse tumor of each of three types: breast, colon, and lung. In July 1983, DEB implemented a project to determine the feasibility of screens based on sequential in vitro and in vivo testing against batteries of human tumors, a battery consisting of ten or more tumors of a specific type. Objectives are to determine the extent to which in vitro assays, using transplantable human tumors grown in athymic mice as the source of tumor cells, are capable of identifying drugs with activity against the same tumors in vivo. The goal is to find drugs for trial against human cancers with the best chance of responding.

Detailed Drug Evaluation

DEB placed additional emphasis on active drugs in studies designed to provide maximum pre-clinical information; e.g., the influence of modifying factors such as drug route relative to tumor site, host tumor cell burden, patterns of cross-resistance to clinical drugs, etc. Bioassay of experimental clinical formulations, standardized route and schedule dependency testing, and biochemical characteristics of drugs in development to the clinic continued.

Route and schedule dependency studies were completed for Tiazofurin, Didemnin B (NSC-325319), and Bactobolin (NSC-325014). Pre-clinical activity of experimental clinical formulations was validated for Caracemide, Isopropyl pyrrolizine (NSC-278214), Ara-5-Ac (NSC-281272), Bisbenzimidazole (NSC-322921), Didemnin B, and Trimetrexate glucuronate (NSC-352122). ADL (CM1-7397) reported on intracellular actions of Mitindomide (NSC-284356), Caracemide, Bactobolin, and Phyllanoside (NSC-328426). ADL (CMO-7302) also investigated the potential antitumor superiority of analogs of active drugs, conducted specialized screening of natural products, and special studies of high priority drugs, and evaluated rationally based drug combinations. The xanthine oxidase inhibitor, Allopurinol (NSC-3590) did not influence the toxicity or antitumor activity of Tiazofurin, an IMP dehydrogenase inhibitor. The study was suggested because Allopurinol inhibits uric acid production while Tiazofurin is likely to increase serum levels of uric acid in humans. This and other drug combination studies were planned in collaboration with the Biochemical Modulators Group, DCT.

ICR, England (CM4-3736) found a marked species difference in rate of DTIC

(NSC-45388) activation by N-demethylation. The low rate of activation in humans resulted in concentrations of active metabolite insufficient to inhibit mouse PC6 cells in vitro. The species difference may account for the low level of DTIC efficacy clinically despite its substantial activity against mouse tumors.

Over the past year, SoRI, CM9-7309, was divided for recompetition into four functional areas: in vivo screening, detailed drug evaluation, tumor quality control, and model development (Table 1, Sections 1, 3, 4, and 5). Detailed drug evaluation tasks included bioassay of formulations, route and schedule dependency tests, and studies of cross-resistance.

Model Development

Mason Research Institute contract, CMO-7325, and model development aspects of SoRI contract, CM9-7309, were recompeted with the aim of awarding one contract for development of the in vitro/in vivo screens discussed earlier. Mason Research (CMO-7325), continued study of the SRC assay as a predictor for clinical efficacy. Four Phase I drugs: NMF, Echinomycin (NSC-526417, ECH), Triciribine phosphate (NSC-280594, TCNP), and HHT were compared with standard anticancer drugs against 60-80 fresh surgical explants of human tumors in the SRC assay. The fraction of responding (>20% regression) tumors of selected types is given below.

Drug	Breast	Lung	Ovarian	Colorectal	Cervical
TCNP	4/19	5/12	7/10	3/8	7/8
HHT	1/9	3/12	3/10	0/8	2/8
ECH	1/7	2/7	4/9	1/6	0/6
NMF	1/7	4/7	2/9	0/6	0/6
CTX	5/19	3/12	5/9	2/7	4/8
5-FU	3/19	-	4/10	1/6	-
MTX	1/18	4/12	-	-	3/8
ADR	2/19	2/12	1/10	-	2/8

Future Plans and National Cooperative Drug Discovery Groups

Projects planned for the near future are incorporated in earlier parts of this report because they seemed appropriate to the subject under discussion. We plan to expand our effort in testing new active drugs in "more challenging assays" as depicted in Figure 1. To this end, the CCS organized a symposium on Cellular Resistance to Anticancer Drugs. Two areas were identified as deserving priority for future research: (1) reversal of resistance to natural products by calcium blockers or other agents; and (2) identification and characterization of human tumor cells showing pleiotropic drug resistance. Recent advances in molecular biology, medicinal and organic chemistry, pharmacology, and biochemistry provide unprecedented opportunities for the design and pre-clinical evaluation of new potentially powerful anticancer therapies. Exploitation of these leads and their extrapolation into new drugs with unique mechanisms of action and into new strategies for cancer treatment will require mobilization of the Nation's most talented and dedicated scientists. To accomplish this we plan to implement National Cooperative Drug Discovery Groups (NCDDG). It is anticipated that each NCDDG will be multi-disciplinary and multi-institutional; that is, it will be

composed of the most creative scientists in academic, non-profit research, and industrial organizations. The goal is to apply strong scientific rationales to the design and synthesis of new anticancer therapies and to the selection or development of the most informative pre-clinical assays for recommending new treatments for human cancer.

Publications by Staff

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2. Abbott, B. J.: In vitro screening systems. Principles of Cancer Chemotherapy (in press).

3. Venditti, J. M.: The NCI antitumor drug discovery program, current and future perspectives: A commentary. Cancer Treat. Rep. (in press).

4. Venditti, J. M., Wesley, R. A., and Plowman, J.: Current NCI pre-clinical antitumor screening in vivo. Results of tumor panel screening, 1976-1982, and future directions. Advances in Pharmacology and Chemotherapy (in press).

TABLE 1

CONTRACTS MANAGED BY DEB AND FUNDING LEVELS,* FY 1983

1.	In Vivo Screening	$3,367,000
	Arthur D. Little, Inc. (CMO-7346)	400,000
	Battelle Columbus Laboratories (CMO-7266)	675,000
	IIT Research Institute (CM9-7316)	700,000
	Institut Jules Bordet, Belgium (CMO-7350)	192,000
	Mason Research Institute (CM9-7317)	700,000
	Southern Research Institute (CM9-7309)+	700,000
2.	In Vitro Screening	$ 737,000
	University of Arizona (CM1-7497)	365,000
	UCLA (CMO-7420)	135,000
	Cancer Therapy & Research Foundation of South Texas (CMO-7327)	149,000
	Mayo Foundation (CMO-7419)	88,000
3.	Detailed Drug Evaluation and Development	$1,161,000
	Arthur D. Little, Inc. (CMO-7302)	(421,000)
	Arthur D. Little, Inc. (CM1-7397)	137,000
	Institute of Cancer Research, England (CM4-3736)	125,000
	Southern Research Institute (CM3-7552)	254,000
	Southern Research Institute (CM9-7309)+	645,000
4.	Model Development	$ 420,000
	Mason Research Institute (CMO-7325)	120,000
	Southern Research Institute (CM9-7309)+	300,000
5.	Tumor Quality Control	$ 300,000
	Southern Research Institute (CM9-7309)+	300,000
6.	Support Services	$ 187,000
	IIT Research Institute (CM9-7213) (six months) Biotech Research Laboratories (CM3-7558) (six months)	187,000
	Total	$6,172,000

*Negotiated or estimated to nearest thousand.
+Multifaceted contract.

TABLE 2

STATUS OF ACTIVE COMPOUNDS

APRIL 1, 1982 - MARCH 31, 1983

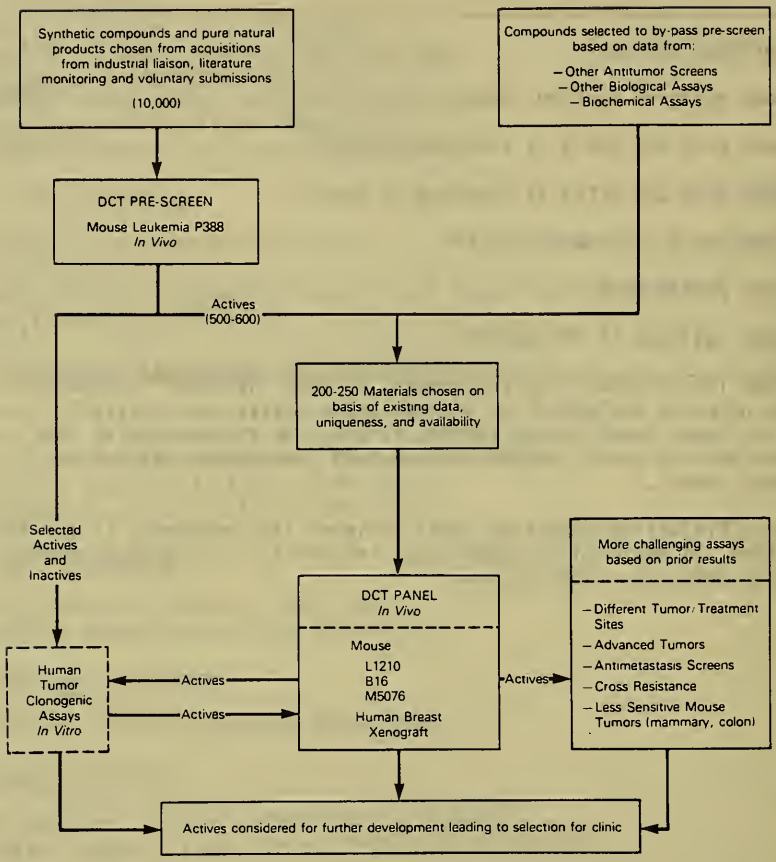
New confirmed actives	674
Compounds assigned to tumor panel	224*
Compounds with DN2 activity referred to DEC+	24
Compounds with DN2 activity referred to Pre-DN	23
DN2A candidates recommended to DN	9
Compounds passed DN2A	8
Compounds assigned to DN special	1

*Includes 190 selected from pre-screen, 22 that "bypassed" pre-screen because of known biological or biochemical activity, ten initially active in human tumor colony forming assays, one recommended by the Platinum Working Group, and one recommended from Project to Review Old Drugs (PROD).

+The Drug Evaluation Committee (DEC) reviewed 110 compounds, 13 for the first time, 36 recycled for additional information, and 61 active congeners of compounds under review.

FIGURE 1

FLOW OF DRUGS THROUGH DCT SCREENS, 1983



ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

The Pharmaceutical Resources Branch (PRB) is structured to provide comprehensive pharmaceutical services to the various Programs of the Division of Cancer Treatment. The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. The Branch supervises a total of 23 contracts consisting of 18 level of effort contracts with a combined annual effort of more than 67 man years and five mission contracts for pharmaceutical production.

The major contract areas include: chemical preparation and pilot plant production; analytical services; pharmaceutical research and development; and pharmaceutical manufacturing. The synthesis and distribution of radiolabeled chemicals and drugs are also provided through PRB contract sources. Additionally, the Branch is responsible for shelf life surveillance, storage and distribution, and computerized inventory maintenance of all drug products used in the Clinical Programs of the Division of Cancer Treatment (DCT).

A formulation research laboratory on the NIH campus is operated and staffed by the Pharmaceutical Resources Branch. This laboratory is assigned research projects of high Program interest which present difficult drug delivery problems. Most of the chemical agents developed by the Laboratory of Medicinal Chemistry and Biology are assigned to this laboratory. This arrangement facilitates a scientific exchange between the developer and formulator and a team approach to the ultimate product design.

Staff

The Pharmaceutical Resources Branch is presently staffed with six senior professionals, one technical and two secretarial personnel. In addition, one visiting associate and one visiting fellow are assigned to the formulation laboratory. The classification of the senior professionals is as follows: four PHS Commissioned Corps Pharmacists; one Ph.D. analytical chemist; and one Ph.D. medicinal chemist.

The Branch consists of three Sections:

1. Chemical Resources Section - Head, Robert R. Engle, Ph.D.

The primary functions of the Chemical Resources Section are to provide for resynthesis, large-scale production and procurement services for the acquisition of chemical substances. These services are accomplished by the management and supervision of a contract program for resynthesis assessment and pilot plant production of various quantities of bulk substances intended for tumor screening panels, preclinical toxicology and pharmaceutical manufacture of clinical investigational products.

Another major function of the Section involves the management of a contract program to prepare and distribute radiolabeled materials. These materials are distributed to authorized investigators for clinical pharmacology and other related studies.

The objectives of the Section are achieved through the management of a contract program for the preparation of various quantities of bulk chemicals and drugs, and the acquisition of commercially available substances through NIH procurement contract procedures.

The chemical and radiolabel preparation laboratories, taken collectively, provide the means of obtaining a broad variety of chemical compounds and the ability of providing large quantities of high purity drug substances. The Section supervises seven chemical prep lab contracts consisting of a combined annual effort of approximately 29 man years. Additionally, two radiosynthesis laboratories are supervised by the Chemical Resources Section with a combined annual effort of seven man years.

2. Analytical and Product Development Section - Head, James C. Cradock, M.S.

This Section has two major functions: (1) the analytical assessment of chemicals and formulated products and (2) the development of investigational pharmaceutical dosage forms for clinical trial.

The analytical component of the Section supervises contractors engaged in the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions, and identification of impurities and/or degradation products. The Section's staff prepares bulk chemical specifications used for acceptance criteria of additional supplies either from commercial sources or chemical preparation contractors. The specifications and validated analytical methodology are prepared in a format suitable for submission to the Food and Drug Administration as part of the NCI's Investigational New Drug Application. The Section is also responsible for chemical evaluation of new investigational dosage forms and for the shelf life surveillance of all formulated investigational products.

The pharmaceutical product development component of the Section is responsible for evaluating all drug substances for formulation approaches. Many substances present difficult formulation problems due to poor solubility and stability characteristics. Since most preparations are intended for intravenous use, studies are undertaken to assess the solubility and stability in a variety of pharmaceutical vehicles. New approaches to enhance solubility are emphasized since few suitable methods are available to the formulator of parenteral products.

Most of the product development effort is conducted under contract with the Section staff serving as project monitors. In addition, an intramural formulation laboratory, supervised and maintained by this Section, is investigating methods to solve drug formulation problems.

The Analytical and Product Development Section is responsible for the supervision and management of eight contracts: three analytical contracts with a combined annual effort of 18 man years; three pharmaceutical R & D contracts with a combined annual effort of eight man years; one combined R & D (one and one-half man years) and pharmaceutical contract; and one shelf life contract involving an annual three and one-half man year effort.

3. Clinical Products Section - Head, Larry M. Kleinman, M.S.

This Section is primarily responsible for the management and supervision of pharmaceutical contracts for the production of investigational dosage forms. The Section is also responsible for the storage and distribution of all clinical products used in the Clinical Programs of the DCT.

The Clinical Products Section manages four pharmaceutical mission type contracts with capabilities to produce a broad variety of pharmaceutical products. The Section also manages a storage and distribution contract with computer capabilities for accurate accountability of the disposition of all investigational products.

In addition, this Section manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this report period, drug purchase expenditures were in excess of 2.6 million dollars. A significant amount of staff time is expended in this area in preparing purchase specifications, award justifications and periodic budget projections throughout the year. Several types of NIH contract mechanisms are utilized for these procurement actions including bids from suppliers, blanket purchase orders, direct purchase contracts, etc.

Investigational product literature in the form of Investigational Drug - Pharmaceutical Data sheets is prepared by the Section. These information sheets are also supplied in bound book form (NIH Publication No. 83-2141) which is updated periodically. During this reporting period, over 2,000 issues were distributed.

Goals and Accomplishments

The goals of the Branch are to provide high quality chemicals and pharmaceutical products to the Programs of the DCT in a timely and efficient manner. The achievement of these goals involve coordination of all chemical, analytical and pharmaceutical disciplines. The Branch functions as a team to achieve its goals and during the 1983 period of this report, a high level of successs was obtained as characterized by the eight new INDs filed in calendar year 1982 and a good start for new INDs in 1983.

During this reporting period, the chemical prep lab contractors prepared over 136 compounds, 23 such drugs totaling 159 kilograms were delivered for pharmaceutical product use. These drugs included Tiazofurin (NSC-286193); Dihydro-5-azacytidine (NSC-264880); Ara-AC (NSC-281272); Methyl G (NSC-32946); ICRF-187 (NSC-169780); and CBDCA (NSC-241240).

In addition, 13 compounds were prepared by the radiosynthesis contractors including N-Methylformamide (NSC-3051); CBDCA (NSC-241240); Ara-AC (NSC-281272); and Tiazofurin (NSC-286193).

The analytical activities in the Branch have experienced the most significant changes due to increased demands from the Food and Drug Administration requiring additional information on low level impurities, validation of analytical systems and preparation reference standards. The analytical contract effort was increased last year from a 14 man year to a 16 man year effort and most recently to an 18 man year effort. The Section has developed analytical specifications for chemicals and formulated products and provides analytical methods for clinial investigators as requested. A new contract activity in the Analytical and Product Development Section involves shelf life surveillance of investigational products. This contract was obtained to provide continuity to the scheduling, reporting, and methodology of shelf life surveillance. This new contract function involved considerable staff time to organize, develop reporting systems, and supervise analytical validation methods. A system is now in place to provide useful shelf life information in a timely manner.

Another area of significant activity and increasing demands has been in product development. The goals of the dosage development activity are to provide technology that may be applied to resolve difficult formulation problems of diverse chemical compounds. The approaches to increase water solubility for intravenous administration are relatively small. During the past year, emphasis has been placed on solvent and emulsion systems. An additional contract with the University of Arizona has just been awarded. The principal investigator is recently from industry and author of articles and chapters in co-solvent approaches to parenteral formulations.

During the last reporting period, several difficult formulation projects were successfully completed. Most notable was Spiromustine (NSC-172112) which is a highly insoluble and unstable compound with interesting antitumor activity. Other dosage forms developed recently include Menogarol (NSC-269148); Caracemide (NSC-253272); and Fludarabine Phosphate (NSC-312887).

The Clinical Products Section continued to provide adequate clinical supplies of investigational products. No drug shortages were encountered and no drug defect recalls were required. The combined contract effort delivered more than one-half million injectable units and over one million oral dosages. The Section prepared the chemical and pharmaceutical portions of six original INDs and submitted numerous IND amendments for added information.

In summary, the Pharmaceutical Resources Branch has maintained a high level of productivity with preparation of chemicals and pharmaceutical products. Contract support was increased in analytical and product development areas. These areas have had increasing demands due to FDA emphasis for more detailed information at early stages of development and the impact of more complex substances for formulation development.

The Branch expects to achieve its goals in the coming year by employing the team concept with frequent staff meetings and progress seminars. No major problems are anticipated at this time.

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4. Poochikian, G.K., Cradock, J.C., and Davignon, J.P.: Heroin: stability and formulation approaches. Int. J. Pharm. 13: 219-226, 1983.

5. Trissel, L.A., Davignon, J.P., Kleinman, L.M., Cradock, J.C., and Flora, K.P.: NCI Investigational Drugs - Pharmaceutical Data 1983. (DHHS) Publ. No. (NIH) 83-2141, 1983, 198 pp.

6. McCarthy, L.E., Vishnuvajjala, B.R., and Flora, K.P.: Cisplatin-induced vomiting in a cat model: antiemetic activity and plasma levels following intravenous, oral and intramuscular administration. In Dewey, W.L. (Ed.): Cannabinoids 82. New York, Academic Press, in press.

7. Wood, J.H., Flora, K.P., Narisimhachari, N., and Baker. C.A.: Dependence of salivary drug concentration on salivary flow rate. Methods and Findings Exp. Clin. Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03584-11 PRB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research in the Development of Dosage Forms of New Antitumor Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

James C. Cradock, Pharmaceutical Resources Branch

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Pharmaceutical Resources Branch

SECTION

Analytical and Product Development Section

INSTITUTE AND LOCATION

NCI,NIH Bethesda, Maryland

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.4	2.4	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project describes the activities of the formulation laboratory of the Pharmaceutical Resources Branch. These studies are directed toward resolving some general problems with the intravenous delivery of antitumor agents but also involve some studies to improve the solubility and stability of selected antitumor agents. Dimethylacetamide, an organic solvent, is used as a vehicle in the formulation of several antitumor agents. The compatibility of this solvent with common plasticizer-containing intravenous administration devices was evaluated using a gas chromatographic procedure to detect diethylhexylphthalate (DEHP). Extraction of substantial amounts of DEHP was not observed at the use concentrations (< 10%). However, high concentrations of this solvent are incompatible with these devices.

The plasma expander hydroxyethyl starch (hetastarch) was evaluated as a potential complexing agent for certain poorly water soluble compounds. This substance is a branched chain carbohydrate used clinically in substantial doses. Other polymers, albumin, povidone etc. have been reported to improve drug solubility. However, only modest (< 10 fold) increases in drug solubility have been observed to date with hetastarch.

Camptothecin is a plant product with good antitumor activity in experimental model and was evaluated clinically as the sodium salt of the lactone. The clinical results were disappointing. However, this compound was reevaluated as part of the PROD activity and the pharmaceutical properties were reexamined. Modest increases in solubility were achieved by solvent-surfactant mixtures. However, a prodrug camptothecin-20-(N,N-diethyl)glycinate HCl was prepared that exhibits reasonable solubility, 5 mg/ml, and equivalent activity with the parent compound in the in vitro P388 assay.

1. Compatibility of Infusion Devices With Pharmaceutical Vehicles (Vishnuvajjala, Flora, Cradock)

The intravenous delivery of several antitumor agents requires the inclusion of certain solvents to achieve adequate solubility for intravenous injection. Since plastic devices are used with increasing frequency in intravenous drug therapy, the compatibility of several solvents with these devices is being examined. Of concern are the physical integrity of these devices when exposed to solvents and the extractability of the plasticizer, diethylhexylphthalate (DEHP). The study began with N-methylformamide, NMF, a solvent currently in Phase I trial as an antitumor agent and has been extended to include dimethylacetamide, a vehicle used in the formulation of several antitumor agents. A gas chromatographic method was developed to detect low levels of DEHP in aqueous solution. The following table lists the amounts of DEHP extracted after prolonged exposure (20-24 hours) to IV sets and flexible PVC infusion bags.

	DEHP ppm	
Solvent	Set	Bag
5% DMA	Not detected	0.06
10% DMA	Not detected	0.09
20% NMF	0.37	0.19
40% NMF	1.87	1.97

At higher concentrations, the amount of DEHP extracted increases logarithmically and neat solvents dissolve tubing after prolonged exposure. However, at use concentrations the levels of DEHP extracted are minor. A dose equivalent to the LD_{50} or 40% NMF would result in a total dose of 0.19 mg of DEHP and 250 ml of 10% DMA would extract 0.06 mg of DEHP. Patients undergoing blood transfusions were reported to receive up to 10 mg per day of DEHP.

2. Studies to Improve Drug Solubility (Tabibi and Cradock).

Methods to increase drug solubility are a continuing need in the development of intravenous formulations. In past years this project has focused on nonionic surfactants and then on emulsions. Hetastarch is approved for IV use as a plasma expander and is administered at high doses. The influence of hetastarch on the solubility of several antitumor agents of widely varying structures including ellipticine, hexamethylmelamine, and adenine arabinoside was evaluated. No substantial (< 10 fold) increase in solubility was observed and this project has been discontinued.

3. Camptothecin (Vishnuvajjala).

Camptothecin is a poorly water soluble plant product that is quite active in tumor models. The sodium salt of the lactone is also active but at about (10 fold) higher doses. Sodium camptothecin received a clinical trial 15 years ago but the results were disappointing. This agent was recently reevaluated by the PROD group and the pharmaceutical aspects were also studied since a number of the current approaches were not in common use in the 1960's. Modest increases were achieved with DMA-cremophor-saline 5:5:90 mixtures

(0.025 mg/ml) that might be minimally adequate for infusion use. A water soluble (5 mg/ml) prodrug was prepared and characterized, Camptothecin-20-(N,N-diethylglycinate) HCl. At slightly acidic pH the compound is adequately stable in solution and exhibits equivalent activity in P388 cell culture system. In vivo antitumor evaluation is in progress.

4. Other Stability Studies (Flora, Cheung, Vishnuvajjala, and Tabibi)

Pharmaceutical aspects of several other compounds have been evaluated during the past year. The published literature contains conflicting data regarding the stability of Azacitidine in infusion fluids and we receive many inquires regarding this aspect from pharmacists and nurses. The pharmaceutically acceptable stability limit (10% decomposition) varied from 2-14 hours. Stability was evaluated by HPLC in four infusion fluids stored in glass or plastic containers at 0.2 and 2 mg/ml. Azacitidine is unstable in all four fluids, particularly in 5% Dextrose Injection (T_{90}=0.8 hours at 0.2 mg/ml), and may be related to the lower pH of that solution. The stability of Azacitidine is essentially the same in Normal Saline, Lactated Ringers and Normasol R with a T_{90} of about 2 hours at 0.2 mg/ml and 2.4-3 hours at 2 mg/ml. The container influenced stability only in the case of 5% Dextrose Injection. The drug was less stable in the plastic bags.

The published data on the stability of Melphalan in IV fluids is minimal and this aspect was further studied using an HPLC method previously developed in this lab. The degradation of Melphalan followed first order kinetics and the rate was dependent upon the chloride ion concentration of the infusion fluid. Acceptable stability (T_{90} at 20°C $\geq$ 4 hours) was achieved in Sodium Chloride Injection (0.154 meq/L Cl^-) but not in fluids containing lower amounts of Cl^-.

Publications

1. Bykadi, G., Flora, K.P., Cradock, J.C., and Poochikian, G.K.: Determination of ellipticine in biological samples by high performance liquid chromatography. J. Chromatogr. 231: 137-144, 1982.

2. Flora, K.P., Cradock, J.C., and Kelley, J.A.: The hydrolysis of spirohydantoin mustard. J. Pharm. Sci. 71: 1206-1211, 1982.

3. Litterst, C.L., Flora, K.P., and Cradock, J.C.: Bioavailability of ^{9}-tetrahydrocannabinol-derived radioactivity following intramuscular administration of ^{9}-11-^{14}C-tetrahydrocannabinol to rabbits. Res. Commun. Sub. Abuse 3: 453-465, 1982.

4. Poochikian, G.K., Cradock, J.C., and Davignon, J.P.: Heroin: stability and formulation approaches. Int. J. Pharm. 13: 219-226, 1983.

5. McCarthy, L.E., Vishnuvajjala, B.R., and Flora, K.P.: Cisplatin-induced vomiting in a cat model: antiemetic activity and plasma levels following intravenous, oral and intramuscular administration. In Dewey, W.L. (Ed.): Cannabinoids 82. New York, Academic Press, in press.

ANNUAL REPORT OF THE TOXICOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

The primary function of the Toxicology Branch is the preclinical toxicologic characterization of potential antineoplastic agents. The agents evaluated are identified by the Decision Network Committee, Developmental Therapeutics Program, on the basis of selection criteria identified in the Linear Array. The Toxicology Branch provides the toxicological information required for the complete, preclinical scientific evaluation of antitumor drugs. The preclinical data accumulated provides the information base for deriving the initial clinical dose and the toxicological base required for an Investigational New Drug Application to the Food and Drug Administration.

The Branch achieves its objectives in preclinical toxicity and safety evaluation through the operation and management of a prime toxicology contract in which the qualitative and quantitative toxicological profiles of antitumor drugs and modalities are determined in animals. For ease in management the Prime Contract is divided into four definitive tasks.

Task I is devoted to the complete preclinical toxicologic evaluation of cytotoxic agents, radiosensitizers, radioprotectors, etc. Standardized protocols using mice, rats and dogs are followed to determine the initial dose for Phase I clinical trials, to verify safety of clinical doses and to evaluate specific target organ toxicity. Task II studies are concerned with evaluation of drugs using a limited series of tests. These studies are performed to complete the toxicity profile on compounds for which some toxicology data is available. Task III involves the development and implementation of in vivo and in vitro tests to evaluate organ specific toxicity. The "special studies" carried out under this task yield important information leading to development of new, more meaningful toxicity testing protocols. Task IV of the Prime Contract deals with the administrative aspects of toxicity testing such as data handling, subcontractor monitoring as required by Good Laboratory Practice Regulations, and financial and program management.

The major responsibility, and the product of the Branch's efforts, is to generate two drug toxicity reports. The first report, Phase I, consists of data from mouse lethality studies and the 'in life' portion (all observations up to necropsy) of dog toxicity studies. This report is submitted to the Food and Drug Administration as Attachment 6a to the Investigtional New Drug Application (INDA). A second, Phase II, report contains all data included in the Phase I report plus data from the rodent toxicity studies and the histopathologic evaluation of tissues from the treated dogs. The Phase II report is filed with the FDA ninety days after the INDA has been submitted.

The following reports have been submitted to CTEP for filing with the FDA in 1983.

Phase I	Date
Tiazofurin (NSC-286193)	11/12/82
Acodazole (NSC-305884)	11/12/82
Caracemide (NSC-253272)	04/06/83
Phase II	
SR 2508 (NSC-301467)	10/15/82
WR 2721 (NSC-296961)	10/15/82
Spirohydantoin Mustard (NSC-172112)	11/02/82
Taxol (NSC-125973)	01/02/83
Fludarabine Phosphate (NSC-312887)	02/22/83
Tiazofurin (NSC-286193)	03/23/83
Acodazole (NSC-305884)	03/28/83
Rat Lethality and Toxicity (x5)	
Spirohydantoin Mustard (NSC-172112)	04/26/83
Acute Cardiovascular Toxicity	
Poly ICLC (NSC-301463)	01/06/83

Preclinical toxicology studies are currently in various stages of completion on Rapamycin (NSC-226080), Menogarol (NSC-269148), Didemnin B (NSC-325319), Bactobolin (NSC-325014) and Bisbenzimidazole (NSC-322921). A sixth compound, Trimetrexate (NSC-352122) is under test at Warner-Lambert using NCI preclinical toxicology protocols. The Toxicology Branch has continually consulted with Warner-Lambert toxicologists and reviewed and evaluated the data. Four compounds, Melphalan (NSC-8806), Indicine-N-oxide (NSC-132319), Pentostatin (NSC-218321), and 5-Azacytidine (NSC-102816) are currently on test in mouse lethality studies using both the x1 and x5 dosing schedules. Information from these tests will provide the Blood Level Working Group with LD10, LD50, LD90 values for comparison with pharmacokinetic data. In addition, Acodazole is currently being tested in the rabbit ear vasotoxicity screen to ascertain dose-response relationships in light of the low level *in vitro* compatability between the drug and blood.

In its continuing efforts on protocol planning and evaluation the Branch analyzed previously acquired data for species versus toxicity relationships. Review of data developed over the past few years indicated that the mouse was an inappropriate animal model for standardized toxicity testing of formulated antineoplastic drugs. Numerous drugs could not be administered to the mouse because of volume constraints; blood samples for hematology and clinical chemistry were frequently inadequate and baseline data for these parameters were extremely variable, thereby making it virtually impossible to analyze the data. Evaluation of the mouse

retrospective studies and data from toxicologic evaluation of new cytotoxic agents were presented to the FDA. A recommendation was made that the optional portion of the NCI protocols include toxicity studies in rats instead of mice. Therefore, the NCI preclinical toxicology protocols now include the following studies:

1) Mouse lethality studies x1 and x5 - human doses extrapolated from these data
2) Dog toxicity studies x1 and x5 - safety of projected human dose established; organ toxicity data developed
3) Rat toxicity studies x1 and x5 - (optional studies) - toxicity data in second species

Alternative protocols have also been developed for cytotoxic agents in which in administration to the mouse has proved to be of little value in lethality studies because of limited drug solubility. These protocols use intraperitoneal administration in the mouse or intraperitoneal or intravenous administration in the rat. All three alternate protocols are currently being used for toxicity testing of drugs with limited solubility.

An agreement was reached between the NCI and the FDA regarding the necessity of longer duration toxicity studies. The Toxicology Branch organized informal guidelines for these studies as the focal point for continuing discussions. It is anticipated that formalized guidelines can be approved and implemented as part of the toxicology program in early FY 1984.

The data handling system (Toxicology Information System - TIS) has been installed in each laboratory and the Toxicology Branch. This significant step in data handling (acquisition/retrieval) minimizes the time required for data collection and distribution, permits rapid assessment, and provides high quality computer generated reports. The TIS will enable staff to readily review all animal toxicology data and make comparisons with Phase I clinical data. This is a critical element in designing long-term toxicity studies on promising antineoplastic drugs.

Two important administrative elements within the Toxicology Branch were addressed this year. Firstly, the Prime Contract was recompeted with two offerors submitting proposals. Following the appropriate source evaluation criteria Battelle Columbus Laboratories was determined as the awardee. The new Prime Contract was fully awarded on July 1, 1983. The second element concerns the appointment of the permanent Chief of the Branch. Dr. Charles K. Grieshaber assumed these responsibilities in April, 1983.

Publications and Presentations

Staff

1. Lowe, M.C. and Davis, R.D.: The current toxicology protocol of the National Cancer Institute. In Carter, S.K. and Hellman, K. (Eds.): Principles of Cancer Chemotherapy. New York, McGraw-Hill, 1983 (in press).

2. Lowe, M.C.: Commentary: Large animal toxicology studies of cancer drugs. In Carter, S.K. and Hellman, K. (Eds.): Principles of Cancer Chemothrapy. New York, McGraw-Hill, 1983 (in press).

Publications and Presentations (continued)

Staff

3. Lowe, M.C. and Davis, R.D.: Use of the mouse as primary species for the preclinical toxicologic evaluation of oncolytic agents: comparison of the results of a National Cancer Institute lethality/toxicity study of twenty drugs with historical data from clinical trials. Cancer Treat. Rep. 1983 (in press).

Contractors

During this year contractors and subcontractors presented 16 papers or abstracts which involved NCI support.

ANNUAL REPORT OF THE INFORMATION TECHNOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

The Information Technology Branch (ITB) has been working on the installation of the new Drug Information System (DIS). The goal of the DIS is to provide DCT staff with online access to all preclinical drug development data via an interactive computer system. This is expected to reduce dramatically the amount of paper used by the current DTP operation, facilitate usage of data, and increase the timeliness of responses to queries.

Work on this task is progressing rapidly and five of the six major DIS modules are now in various stages of implementation. The sixth, with which DCT orders samples of chemicals to be tested, is already in operation, and this has allowed the program to curtail the contract which previously had monitored the inventory of chemicals. As new DIS capabilities become available, cost savings are beginning to appear in a variety of areas, such as the chemical database contract. Meanwhile, the amount of searching being conducted by DCT staff, particularly the chemists and biologists, is increasing rapidly as searching becomes less dependent on ITB intervention and thus more convenient. Most of the files in the DIS are designed to be automatically updatable as often as daily. Thus currency is maximized with little staff effort.

The ITB collaborates actively with other programs within DCT and elsewhere. In this connection, the Branch has developed toxicity databases which are used by various other groups and it has also produced a variety of technical reports on subjects of interest to DCT scientists. In collaboration with Lederle Laboratories, a structure-activity relationship study of anthracyclines has been completed and, together with the NCI Division of Cancer Cause and Prevention, ITB has made the database of Chemical Carcinogens, many of which are of interest to DCT as cytotoxic agents, generally available in a search system. In addition, an effort is being made to produce and disseminate a publicly accessible version of the NCI antitumor testing database without infringing upon the numerous confidentiality agreements that exist between NCI and suppliers of chemicals.

In connection with its charge to apply modern computer capabilities to DCT problems, ITB has brought the computer terminal into DTP Committee meetings, both to display data and also record decisions. The Branch is also working to capitalize upon modern technologies such as the laser printer, which can print reports containing chemical structures and other graphics such as letterheads and signatures; the use of barcodes to label samples; and a teleconferencing system which is now being used to conduct technical meetings with contractors, thus eliminating the travel expenses associated with such meetings.

Office of the Chief, ITB

This Office supervises and coordinates the activities of the two Sections of the Branch, directs the development of the new Drug Information System (DIS), and initiates and pursues appropriate information activities with other units within NCI and NIH, other Agencies of the Government and private sector organizations.

1. Development of the Drug Information System (DIS)

Implementation of each of the six major modules of the DIS is now well advanced. A timetable has been established with a working completion date of 2/84. Currently, work is on schedule.

Termination in 3/83 of the services contract between DEB and IITRI necessitated early installation of the DIS Ordering module. Recording of the minutes of the DTP Prescreen Committee meeting is now automated and orders from these minutes flow directly to the order module. Order and receipt of chemicals is recorded by the computer and both the Ordering and Inventory files are adjusted appropriately. A "QNS report" on all outstanding orders is available online to DTP staff and is also printed automatically every Sunday, immediately following the database update which is clock-triggered and runs without operator intervention.

2. Automation of Committee Meeting minutes

Transactions of the Prescreen Committee are now captured by computer. A projecting computer terminal is used to display on a wall screen the structures or data under discussion. The minutes are provided to each Committee member within an hour of the end of the meeting and acquisitions which are based upon Committee decisions are passed within the computer to the Order module.

3. Screening Protocols

Summaries of the new screening protocols are being prepared automatically for use in the DIS as online 'HELP' messages.

4. Use of Pattern Recognition Techniques

A study, carried out jointly with Lederle Laboratories, of the relationships between the structure of anthracyclines and their antitumor activity has been completed.

5. Public Release of NCI Data

An Interagency Agreement calling for public release of open screening data has been drafted between NCI and the National Technical Information Service (NTIS) and is awaiting approval by both agencies.

6. Project to Review Old Drugs (PROD)

Data were collected and summarized for some 30 PROD candidates. A file is being maintained for each of the 4 DN2A PROD compounds.

7. Acute Toxicity Data

The ability to develop acute toxicity information from screening data has been established and has been used in a number of projects such as the Human Tumor Colony-Forming Assay study.

8. ITB Reports

ITB Staff has prepared 11 reports on subjects ranging from instructions for online retrieval of inventory and screening data (prepared for DEB) to an analysis of all metal-containing compounds in the master database (prepared for DCCP).

9. Chemical Carcinogenesis Database

In collaboration with DCCP, development has been completed of the Chemical Carcinogenesis Research Information System (CCRIS). The file has been made interactively searchable and added to the NIH-EPA Chemical Information System at DCCP expense. It was released for public use in 7/83, and is being used heavily.

Biological Information Section, BIS

The Biological Information Section maintains the flow of screening data into the master database, responds to requests from DCT staff for information from the database for use in decisions regarding continuation or termination of testing, and is responsible for the design and implementation of the biology-related modules of the DIS.

1. Screening Database

Maintenance of this large database has proceeded with a view to its forthcoming use in the online DIS. The Master database, partitioned into an "Active File", an "Archive" and a "Deep Archive", has been reorganized so that every test line (dose-response) is now uniquely identified internally by the computer, thus permitting the DIS programs to link any test line with the correct NSC number. Finally, a considerable amount of work has been done on the adjustments that will become necessary when data from two NSC numbers representing the same compound are merged (see CIS #3, below).

2. Reports to DTP

In order to encourage hands-on searching of the DTP files, online access to both Inventory data and Screening Data Summary Reports has been provided to DTP staff this year. Both have been well accepted and used; over 60 SDS reports, hundreds of structure retrievals,and several thousand Inventory reports were generated with no ITB intervention.

The willingness of DTP staff to do their own searches in this way has relieved ITB staff of much of the burden of searching on demand. In spite of this however, 265 ad-hoc queries were dealt with by ITB this year - an increase of 18% over last year, which signals a large overall increase in searching requirements. In addition to this, routine weekly and bi-weekly reports on the Human Tumor Colony-Forming Assay have been provided to DEB.

In view of DEB's experience with urgent Congressional or Freedom of Information Act-related requests for information concerning contracts, an interactive indexing system has been developed, permitting location of specific contract reports.

Interactive computer programs have been developed to enable NPB to build and maintain a searchable file of plant and fermentation products. The NPB also requested and received routine reports, including the (monthly) Material of Interest report, and the (quarterly) P388/L1210 report.

Chemical Information Section, CIS

The Chemical Information Section provides data processing support in the area of chemistry and is also responsible for design and implementation of the chemistry-related modules of the DIS.

1. Input of Chemical Structures

An efficient structure-input program, now being tested, allows entry of complex organic structures correctly in under 3 minutes and should lead to major cost savings. This program generates a a connection table and a vector diagram from the entered structure, and indexes and stores both for subsequent searching and display, respectively. It will be the foundation of the DIS Pre-Registry module, whose completion is scheduled for 11/83.

2. Output of Chemical Structures

The NCI need for reports which incorporate chemical structures continues to become more pronounced. The program which generates such reports has been improved considerably and a serious indexing problem has been corrected. In addition, structures may now be positioned on the page at the user's discretion - a minor, but useful feature. Significant optimization of the program was also effected and it now runs at about 20% of its earlier cost.

The long range solution to all problems relating to the inclusion of structures in reports lies in the acquisition of a high-speed, laser beam printer. Specifications have been developed and a procurement action is now well advanced for such a printer, which will replace the two existing units in the Blair Building.

3. Review and Correction of Existing Chemistry Data

The Chemistry files are being reviewed and corrected prior to their being merged into the DIS. Approximately 4,000 chemicals have more than one NSC number. There is no chemical basis for this in some 75% of the cases and these data are being merged under a single NSC number. The data on as many as 40% of the Chemcards are incomplete or inaccurate and these data are being corrected. Finally, name and synonym information for over 30,000 compounds, including all the SAC file, have been added to the database.

4. Use of Computers in Committee Meetings

A computer terminal which can project images onto a wall screen has been acquired and installed in the Blair Building Conference Room. It permits the retrieval and display of data and structures (singly, or in pairs, e.g. parent and analog) and has proved to be of value to the Prescreen Committee and also in training users of the computer systems.

5. Use of Barcodes

The DIS is designed to generate and use machine-readable barcodes to label samples. The design of a sub-system to generate and read barcoded labels is complete, the necessary hardware has been ordered and implementation is expected to be complete by 11/15.

6. Teleconferencing

The use of Teleconferencing has been adopted by the Branch. Video communication has been tried and found to be relatively costly and only marginally useful. Consequently, the Branch is currently using audio-only facilities. The cost savings that have ensued are noteworthy. A single one-day meeting with Chemical Abstracts Service staff in Columbus, OH., for example, costs over $300 per person in travel expenses. The monthly rental cost of the teleconferencing equipment at each location is $87.00.

Publications

1. Milne, G. W. A., Development of a Chemical Information System. J. Assoc. Off. Anal. Chem. 65: 1249-1258, 1982.

2. Milne, G. W. A.: Chemical Information Systems. Proceedings of the 6th. International Conference on Computers in Chemical Research and Education. In Heller, S. R. and Potenzone, R. (Eds.). Amsterdam, Elsevier, 1982, 45-60.

3 . Heller, S. R., Budde, W. L., Martinsen, D. P., and Milne, G. W. A.: The NIH/EPA Mass Spectral Data Base and Search System. Int. J. Mass Spectrom. Ion Phys., 47: 313-316, 1983.

4. Milne, G. W. A., A Computer System for Use in the Review of Old Drugs. Med. Pediatr. Oncol., in press.

ANNUAL REPORT OF THE EXTRAMURAL RESEARCH AND RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

Description

The primary function of the Extramural Research and Resources Branch is to administer extramural research supported by grants for preclinical development of antineoplastic drugs which act specifically or selectively against malignant growth with minimal toxicity to the host. The major areas of emphasis in the Biochemistry and Pharmacology research program are: drug design and synthesis, natural products development, experimental therapeutics, selective screening for activity, comparative pharmacology and toxicology and mechanism of drug action. This Branch maintains liaison and coordinates its research activities with those of other Divisions of the National Cancer Institute, other Federal agencies, and academic institutions at the national and international levels. As new findings and significant developments occur, appropriate changes are made in programmatic emphasis and support in each of its major research categories. Periodically, the Branch staff also establishes short- and long-term preclinical drug research guidelines to assist peer review groups and other program staff in evaluating the relevance of proposed research activities to goals of the Developmental Therapeutics Program.

During Fiscal Year 1983 the Branch supported 314 research projects totalling $34.9 million dollars. The distribution of projects among the major categories reflects efforts to ensure a balanced research program in accordance with the need and current priorities for drug development.

BIOCHEMISTRY AND PHARMACOLOGY PROGRAM
BY SUB-CATEGORY
FY 1983

	Number of Grants	Total Amount (Thousands)
SYNTHESIS & CHEMISTRY	121	$ 11,917
NATURAL PRODUCTS	30	2,324
SCREENING & EXPERIMENTAL THERAPEUTICS	28	2,884
COMPARATIVE PHARMACOLOGY	19	1,850
OTHER PRECLINICAL ASPECTS	9	978
MECHANISM OF ACTION	101	9,948
PROGRAM PROJECTS	6	5,014
TOTAL	314	$ 34,915

Significant Recent Results

Inhibition of Metastasis by Proteinases

One of the major problems in clinical and preclinical treatment research is the control of secondary tumor growth by metastasis. A variety of proteinases, including fibrinolytic and thrombolytic types, are present in elevated levels in malignant cells. Proteinases appear to play a major role in the movement of neoplastic cells by catalyzing the degradation of protein in tissues surrounding both primary and secondary tumor sites. A promising approach to the problem of metastasis involves the synthesis of peptidyl aldehyde transition-state analog inhibitors which are highly selective against serine and cysteine proteinases. In vitro tests for proteinase specificity are conducted in order to further refine and to enhance inhibitory action. A metastatic tumor model utilizing mouse B16 melanoma cells in vivo provides information on effective concentration levels of the synthetic inhibitors. These studies also serve to identify sequences of active proteinase in each step of the metastasis and provide guidance on means to control scission and migration of cells from the primary tumor (CA 34530, Schultz).

Natural Products Research

Taxol, a promising chemotherapeutic agent selected on the basis of its activity against the MX-1 mammary, B16 melanoma and CX-1 colon tumor test systems is entering preclinical toxicity studies. Recent research on the mode of action of taxol indicates that it binds to specific receptor sites on the microtubule cytoskeleton of cells and prevents depolymerization of the tubulin molecules. Taxol promotes the number, rate and extent of tubulin nucleations in vitro and stabilizes microtubules in vitro and in cells. Cell replication is arrested in the G_2 and M phases and migration of mouse 3T3 fibroblast cells is inhibited, possibly because polymerization and depolymerization of the cytoskeleton is essential for the cells to distinguish between anterior and posterior surfaces.

The mechanism of action of taxol appears to be unique among those of known chemotherapeutic agents. Research is in progress to elucidate its precise role in regulating cellular functions and to provide guidance for the development of improved drugs (CA 15714, Horwitz).

Cell Kinetics-Directed Treatment Schedules

Considerable research effort is directed at the means of manipulating tumor cell cycle kinetics to achieve maximum cell kill by phase-specific agents. Solid tumors contain relatively low growth fractions. Therefore, agents which induce cell cycle phase synchrony or those that direct non-dividing cells back into the cell cycle for therapeutic attack are valuable adjuncts in combination chemotherapy.

In recent experiments, the compound 1,2:5,6-dianhydrogalactitol (DAG) was tested in Chinese hamster ovary cells (CHO), human adenocarcinoma of the stomach and an Ehrlich ascites tumor system in vivo. In each case, transient, reversible blocking in the S phase was followed by several-fold increases of cell fractions in the G_2-M phases. Subsequent to DAG treatment in CHO cells, bleomycin, an agent most effective in the G_2-M phases, was administered.

At 30 hour post-DAG treatment, cell survival had decreased 300% from that observed when bleomycin was used as a single agent.

Further studies on enhancement of therapeutic response are planned using agents which induce kinetic changes combined with cycle specific cytotoxins (CA 15397, Barranco).

Adriamycin Cardiotoxicity

Adriamycin is one of the most effective anticancer drugs available for clinical use; however, its use in patients is limited by drug related cardiac toxicity. Damage to the cancer cells and the cardiac cells appears to be caused by a common mechanism of drug intercalation to the DNA molecule followed by inhibition of strand replication.

Recent findings indicate that studies of differences between normal and cancer cell membranes may provide more effective leads to selective toxicity than development of rescue agents. Adriamycin was found to be toxic to L1210 cells under conditions in which no free drug could enter the cell. By coupling the adriamycin molecule to insoluble agarose beads, it was possible to prevent the drug from passing through the cell's plasma membrane. Thus cell toxicity apparently results solely from drug action at the cell surface. Although the exact mechanism of drug action is not known, future research should reveal how and why membrane surfaces of malignant and normal cells differ in their composition. Further research will concentrate on developing surface-specific agents in order to spare normal cells the metabolic damages which occur as a result of administering the drug in its free form (CA 24955, Tritton).

Mitochondria as Targets for Therapy

Mitochondria, which serve as energy supply centers for the cell, undergo dramatic morphological changes following administration of anti-cancer drugs. The study of living organelles is limited by current experimental techniques which involve fixation with resultant damage to cells. Recently, Rhodamine-123 (Rh-123), a fluorescent permeant cationic dye that can be applied under nontoxic conditions, was found to accumulate selectively in the mitochondria. Changes in mitochondrial morphology induced by anti-cancer agents correlated closely with irreversible loss of Rh-123 uptake and loss of clonogenic ability. Rh-123 also exhibited anticarcinoma activity *in vivo*. Research is now directed to testing the activity of this compound against epithelial tumors originating in the breast, lung, colon and pancreas. The selective toxicity of Rh-123 to carcinomas may be a characteristic of other rhodamine analogs and other families of permeant drugs. Rh-123 may also provide a convenient alternative to the clonogenic assay as an *in vitro* test that may predict *in vivo* activity. By facilitating studies of mitochondria of normal and malignant tissue, it should be possible to develop new agents which may act specifically against epithelial tumors (CA 33847, Bernal).

Areas for Future Emphasis

Mechanism of Membrane Transport

An understanding of the factors involved in differential cytotoxicity and selectivity of antifolate drugs is of high programmatic interest. Therapeutic

efficacy of these agents is strongly influenced by membrane transport and resulting intracellular levels of unbound drugs. Recent leads in biochemical knowledge of transport mechanisms of methotrexate will be followed to delineate the role of transport mechanisms in other drug-cell interactions and its effectiveness in enhancing chemotherapeutic response.

Combination Therapy

Development of new multi-drug regimens based on precise understanding of pharmacokinetic and biochemical parameters of individual tumor entities remains a high priority item for projected research in the program. The selection of a specific combination for drug synergism as well as optimal dose scheduling data will be based on a rational basis for more predictable correlation in clinical treatment. Research in this area will lead to improved responses by eventual tailoring of multi-drug chemotherapy to fit the characteristics of the tumor under treatment in the individual patient.

Chemotherapy of Tumor Metastasis

A major problem in clinical oncology is the lack of effective chemotherapeutic approach to treatment of metastatic cancer. The promise of adjuvant immunotherapy and chemotherapy of metastatic cancer is based on reducing total body burden of tumor stem cells by surgical removal, leaving the residual cells within the curative potential of available chemotherapy. Aggressive treatment with appropriate supportive care by combination or sequential therapy has been shown to be a feasible approach. Emphasis will be placed on these and other therapeutic concepts to be tested further for greater progress in this area of clinical significance.

Other Program Activities

This program supported the following conferences in FY 1983:

1. A symposium on anticancer and antiviral agents held in Birmingham, Alabama, November 3-5, 1982, organized by Dr. William Niedermeir, University of Alabama, Birmingham, Alabama. Topics included triazenes as anticancer agents, recent work on nucleoside analogs, improved antifolate therapy and development of drugs for cancer patients with viral infections.

2. A conference on platinum complexes in cancer chemotherapy, held in Shelburne, Vermont, June 22-24, 1983, organized by Dr. Irwin H. Krakoff, University of Vermont, Burlington, Vermont. Leading researchers discussed biochemistry, pharmacology, toxicology and development of new platinum complexes and new therapeutic uses of platinum and other metal complexes in cancer treatment.

3. Annual Gordon Research Conference on chemotherapy of cancer, held in New London, New Hampshire, July 25-29, 1983, organized by Dr. Gerald C. Mueller, University of Wisconsin, Madison, Wisconsin. Conference sessions and discussions were devoted to oncogene research, preconditioning regimens in chemotherapy, immune cell modulation and therapy of small cell carcinoma of the lung.

ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

OCTOBER 1, 1982 to SEPTEMBER 30, 1983

Efforts in the Laboratory of Chemical Pharmacology, DTP, DCT are concerned with conducting research, teaching and training, contract administration, and providing administrative and scientific support to the Developmental Therapeutics Program and the Division of Cancer Treatment. The primary research activity of the Laboratory of Chemical Pharmacology is the study of new and established antitumor agents. These studies are chiefly concerned with elucidating the pharmacological properties of such agents, and include an evaluation of their disposition and metabolism, their mechanism of action, the mechanisms by which tumor cells become resistant to them, and their potential adverse effects. Studies are conducted not only in laboratory animals, but also in humans, and the test compounds include antitumor agents as well as other foreign compounds used as models for defining specific processes or mechanisms. Of special concern in the disposition studies of new antitumor agents is the tissue distribution of the compound, including its ability to cross the blood-brain barrier; in addition, the lymphatic absorption of the compound, its biotransformation and its rate of elimination are evaluated in parallel with attempts to develop a model for its pharmacokinetic behavior. These studies require in many instances the development of new analytical methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition and metabolic fate is not fully understood. Other studies in the Laboratory of Chemical Pharmacology are concerned with the development of novel approaches and combined modality regimens for tumor therapy, the rational design of new antitumor agents, evaluation of chemotherapeutic agents in various model tumor systems, and characterization of the transport properties of the blood-brain-CSF system. Efforts are also underway to characterize the toxicity of new antitumor agents, and to develop methods of reversing their toxicity.

A major thrust of research within the Laboratory is directed toward understanding the mechanism(s) of selective toxicity of antitumor agents. Emphasis is placed on elucidating the importance of metabolic reactions that alter the cytotoxic effects of drugs and on endogenous factors that affect the selective toxicity of antitumor compounds. To study the mechanism of selective toxicity of a compound, one must use *in vivo* systems and those *in vitro* systems that can approximate *in vivo* conditions. We have spent a considerable amount of effort developing such systems. Our first priority is to study the reaction *in vivo*. When this is not possible we develop methods to study enzyme reactions in the intact cell or intact organ so as to maintain the microenvironment of the biologic process under study as close to the *in vivo* condition as possible. Research from this Laboratory (see previous annual report) has shown that the isolated perfused rat liver exports uridine at concentrations similar to that found in plasma. Furthermore, plasma appears to be an important source of preformed pyrimidines that can be utilized by cells with an intact salvage pathway and thus could be an important factor determining the effectiveness of certain antimetabolite antitumor agents.

We found, for example, when cultured L1210 cells are incubated in various constant concentrations of uridine, that concentrations of uridine equivalent to that found in the plasma substantially inhibited de novo pyrimidine biosynthesis. The salvage pathway was less sensitive than the de novo pathway to intracellular uracil nucleotide concentrations indicating a preferential use of salvage over de novo synthesis. Furthermore, plasma concentrations of uridine reversed the growth inhibitory effects of PALA in cultured L1210 cells. Thus, manipulation of circulating nucleoside concentrations could be an important method of enhancing the selective toxicity of antimetabolites. Studies were extended in the following areas: (a) manipulation of the donor function of the liver to increase or decrease biosynthesis of salvageable pyrimidines; (b), evaluation of the relative dependencies of cells on de novo vs. salvage pathways for pyrimidine biosynthesis in vivo; (c), synthesis and evaluation of inhibitors of uridine kinase to block the salvage pathway; and (d), regulation of de novo pyrimidine biosynthesis in isolated hepatocytes and the inter-relationship of this pathway with enzymes of the urea cycle.

Techniques to monitor the flux through the de novo pyrimidine pathway in tumors and host tissues in vivo were refined and extended (see previous annual report). This method quantitates the incorporation of pathway precursors (labeled with stable isotopes of carbon and nitrogen) into tissue uracil nucleotide pools by GC/MS. It allows, for the first time, an evaluation of differential effects of inhibitors of de novo synthesis in normal vs. tumor tissues in vivo. PALA, a known inhibitor of de novo synthesis, was used as a test compound and $^{13}CO_2$ was used as the labeled precursor. Pathway flux was inhibited for at least 48 hrs. in Lewis lung carcinoma, a PALA-sensitive tumor line; whereas, in L1210, a PALA resistant tumor line, recovery of pathway activity occurred at 12-24 hrs. This recovery of flux did not correlate with the recovery of aspartate transcarbamylase activity. Studies are underway to study dose-related onset of inhibition and duration of inhibition of a series of de novo inhibitors in normal and tumorous tissues. The formation of dilabeled uracil (through the use of ^{15}N-glutamine or $^{15}NH_4Cl$) permitted the calculation of the total amount of product formed by the pathway in isolated rat hepatocytes. Preliminary studies indicate that such a determination is also possible for in vivo application.

A search for inhibitors of uridine salvage yielded two highly active compounds. One compound is a newly synthesized nitropyrimidine. Resolution of the optical isomers of this nitropyrimidine demonstrated that the salvage-inhibitory properties reside in only one of the isomers. This compound is the first uridine derivative to block uridine phosphorylation in intact cells. Studies are in progress with this compound to evaluate possible therapeutic enhancement of inhibitors of de novo pyrimidine biosynthesis. The other active compound is tiazofurin (NSC-286193) an inhibitor of purine biosynthesis. Both compounds were found to block the utilization of uridine by cultured L1210 cells.

Studies of the hepatic regulation of circulating purines were continued. The role of the liver as a supplier of purines to other tissues for salvage has long been an accepted premise. The results of Pritchard et al. (Am. J. Physiol. 229: 967-972, 1975) indicated that the liver removes hypoxanthine from the plasma and exports adenosine. With the development of new purine analogues as antitumor agents, we initiated studies in the isolated rat liver to determine the role of this organ as a supplier and regulator of purines that could modify the toxicity of these analogues in purine-requiring organs and tumors.

Purine bases and nucleosides were quantitated by HPLC analysis and metabolic interconversions of added purines were determined by radiotracer methodology. Hypoxanthine and inosine were rapidly metabolized to uric acid and approximately 2% of each of these tracers was salvaged by the liver and incorporated into liver purines. Adenosine was rapidly deaminated and the resulting inosine metabolized to uric acid. Adenosine was salvaged to a greater extent by the liver with 20% of the total radioactivity being incorporated into liver purines. Adenine was found to be exported by the liver in increasing concentrations until it reached 1 μM and then plateaued. No adenosine was found in the effluent of the perfused liver, even when the liver was exposed to a 10 μM concentration of hypoxanthine. Thus, it appears that the liver exports adenine and not adenosine as had been previously postulated. Experimentation is underway to explain the discrepancy between our results and those of Pritchard et al. and to determine the importance of adenine export by the liver as a modifier of the action of purine antimetabolites.

Studies were continued on the in vitro and in vivo cytotoxicity of 2'-deoxy-5'-azacytidine (DAC). In vitro cytotoxicity studies with DAC indicate a maximum cell kill of 3 to 4 logs is achievable with optimal conditions of concentrations and exposure time. In vivo cytotoxicity studies with DAC indicate log cell kills of greater than 7 logs at optimal doses. Reasons for this discrepancy are being studied. Advanced L1210 tumor in mice, with late treatment, indicate drug resistant cells (deoxycytidine kinase poor mutants) may be a major problem with DAC. Collaterally sensitive drugs such as cytoxin, BCNU, dihydro-5-azacytidine, 3-deazauridine and tiazofurin are being considered as potential solutions to this drug resistance. Biochemical modulation of DAC activity occurs with thymidine and imune modulation occurs with pyran copolymer.

A basic understanding of the movements of drugs and physiological materials between blood, brain, CSF, and systemic tissues is essential for an appreciation of normal CNS and systemic functions as well as the changes in these transport systems caused by various CNS and systemic malignancies. Sound experimental methods must be used to perform scientifically reliable transport studies. Therefore, various improved techniques for measuring and analyzing transport phenomena have been developed. Among these developments are: (1) better methods of determining blood-brain and blood-tissue transfer constants for materials of low to moderate permeability; (2) a quantitative and more relevant assessment of drug delivery and uptake by normal and tumorous tissue in terms of tissue extraction fractions and microvascular permeability-surface area products; and (3) double-label quantitative autoradiographic and co-imaging techniques to facilitate regional tissue analysis and correlation. These various techniques have been expanded to include regional analysis of systemic tissues as well. Several rat tumor models were examined with these techniques including the ENU-induced oligodendroglioma, the RT-9 intracerebral and flank gliosarcomas, and the ASV-induced astrocytoma. Misonidazole delivery in the RT-9 flank tumor was mainly limited by blood flow. Intraperitoneal administration of test solutes yielded good delivery throughout the peritoneal cavity but lesser penetration into the intestine than the abdominal wall and diaphragm. Intra-arterial drug infusions achieve increased exposure of the target organ and decreased exposure of other tissues when drug is rapidly

metabolized, blood flow through the infused artery is slow, and infused drug is rapidly broken-down in the target organ. For most small brain tumors, blood flow and transcapillary influx (the two major components of intravascular drug delivery) were similar to that of normal brain; however for large brain tumors, blood flow was generally reduced but transcapillary influx ranged from normal to greatly increased.

The preceding outline summarizes the objectives of the Laboratory of Chemical Pharmacology and describes some of the research carried out within this Laboratory during the year. The bibliography for the Laboratory as a whole is listed below, followed by the individual Project Reports which describe this research in greater detail.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CM03500-19 LCHP

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Distribution of Drugs Between Blood, Brain, and Cerebrospinal Fluids

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.0	5.5	3.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Drug delivery systems of the brain, brain tumors, plus other tissues and tumors were studied. Single-and double-label and whole-body quantitative autoradiography were the major techniques employed. The rat tumor models examined included the ENU-induced oligodendroglioma, the RT-9 intracerebral and flank gliosarcomas, and the ASV-induced astrocytoma. For most small brain tumors, blood flow and transcapillary influx (the two major components of intravascular drug delivery) were similar to that of normal brain; however for large brain tumors, blood flow was generally reduced but transcapillary influx ranged from normal to greatly increased. Misonidazole delivery in the RT-9 flank tumor was mainly limited by blood flow. Intraperitoneal administration of test solutes yielded good delivery throughout the peritoneal cavity but lesser penetration into the intestine than the abdominal wall and diaphragm. Intra-arterial drug infusions achieve increased exposure of the target organ and decreased exposure of other tissues when drug is rapidly metabolized, blood flow through the infused artery is slow, and infused drug is rapidly broken-down in the target organ.

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Regional blood flow (F) was measured in ASV-induced brain tumors in rats. Blood flow was variable in individual as well as different tumors; tumor F did not correlate with histologic classification, tumor size, intraparenchymal location, cell density or specific cytological characteristics. Low values of F did correlate with tumor necrosis and hydrocephalus; high values of F correlated with tumorous invasion or association with choroid plexus. Mean tumor F was not significantly different from that of the same anatomic, tumor-free brain region of the contralateral hemisphere (CBA) but F in brain tissue adjacent to the tumor was significantly depressed. Depression of F was observed in tumor-free cortex and corpus callosum.

Regional blood-to-tissue transport of ^{14}C-alpha-aminoisobutyric acid (AIB) was measured in ASV-induced rat brain tumors and expressed as a unidirectional transfer rate constant (K). The magnitude of K was variable and did not correlate with histological classification or specific features of the tumors. Averaged mean K was highest for anaplastic astrocytomas and lowest for gemistocytic astrocytomas (GA); however, the range of measurement within the individual tumor classifications (except GA) was broad. K was not consistently related to tumor size or tumor location; choroid plexus and subependymal tumors were exceptions and had higher K values. The averaged mean K in brain adjacent to tumor was greater than in CBA and generally 1/3 to 1/2 of the value in tumor periphery indicating that these tumors affect the permeability characteristics of adjacent brain capillaries. Estimates of the fracional extraction (E) of AIB by the tumors ranged between 0.009 and 0.2 and indicated that tumor capillaries are not freely permeable to this solute.

An animal model and two different radiolabeled isotopes of 2-deoxy-D-glucose ([1,2-^{3}H]-2-DG, [1-^{14}C]-2-DG) were used to illustrate experimentally for the first time the principles which govern the intra-arterial chemotherapy delivery advantage for the treatment of malignant tumors. Both the delivery advantage to the tumor and the protection to systemic tissue obtained from the intra-arterial infusion into a target artery were measured. The data specifically demonstrates (1) that the maximum intra-arterial delivery advantage is defined in the first passage of isotope through the arterial blood supply to the target issue and is limited by the ratio of cardiac output to carotid artery blood flow (estimated at 30 in the rat); (2) the delivery advantage is reflected in a

5-fold increase in the uptake of the intra-arterially administered isotope in the brain compared to the intravenously administered isotope; and (3) both the intra-arterial delivery advantage and the protection to systemic tissue are dependent on the isotope half-life in blood and the irreversible removal from blood of the intra-arterially administered isotope during its first pass through the brain and/or lungs.

Regional blood flow (F) was measured in RT-9 experimental brain tumors. Blood flow was variable within tumor tissue and the range of F increased with increasing tumor size; the overall range was 6 to 138 ml/100 g min and the maximum range within an individual tumor was 55 ml/100 g min. In all but one case, mean tumor F was less than that in the same antomic region of the contralateral hemisphere (CBA). The magnitude of F was lower in the geometric centers than in the periphery of medium and large size tumors. Brain adjacent to tumor (BAT) had higher F's than the tumor periphery; generally F in BAT was less than that in the CBA. A global depression of F was observed within tumor free cortex and corpus callosum of the hemisphere ipsilateral to tumor implantation and primary growth suggesting a hemispheric reduction in metabolic and functional activity.

Regional blood-to-tissue transport, (K), was measured in experimental RT-9 brain tumors using ^{14}C-alpha-aminoisobutyric acid (AIB) and quantitative autoradiographic techniques. K was variable within tumor tissue and depended on tumor size, location (intraparenchymal, meningeal, or choroid plexus associated), and to a lesser extent on necrosis and cyst formation. Brain adjacent to tumor had higher Ks, particularly around larger tumors, than corresponding brain regions in the contralateral hemisphere. Estimates of the fractional extraction (E) of AIB by intraparencymal tumors were between 0.008 and 0.4. Values of E in this range indicate that tumor capillaries are not freely permeable to this solute. The experimental data suggest that the permeability characteristics of the microvasculature in small RT-9 tumors are similar to that of the host tissue, whereas the microvasculature of larger RT-9 tumors is influenced more by intrinsic tumor factors.

Regional blood flow (F) was measured in experimental (ENU-induced) brain tumors. A total of 15 oligodentrogliomas (ODG), 16 mixed gliomas (MG), 1 astrocytoma, 1 ependymoma and 3 malignant schwannomas were studied in 9 rats. The averaged mean tumor F for all glioma classifications were similar. Flow was fairly uniform within individual ODGs and there was no apparent correlation between F and tumor size or location. The MGs were larger in size than the ODGs and had a wider range of F. Small focal areas of necrosis were observed in 7 MGs, and low flows were usually measured in these regions. Small tumor regions with increased vascularity - frequently with endothelial cell proliferation - were observed in ODGs and to a greater extent in MGs; these regions correlated with small elevations in F in comparison to surrounding tumor tissue. Brain adjacent to tumor usually had higher Fs than that in tumor periphery.

Regional blood-to-tissue transport (K) was measured in experimental primary (ENU-induced) brain tumors using ^{14}C-alpha-aminoisobutyric acid (AIB). A total of 16 oligodendrogliomas, 4 mixed gliomas, 3 astrocytomas, 2 diffuse gliomatoses, 1 anaplastic astrocytoma, 1 ependymoma and 4 malignant scwannomas were studied in 9 rats. The averaged mean K for all glioma classifications was

similar or only slightly higher than a comparable region of brain tissue in the contralateral hemisphere. Values of K varied minimally in the intracerebral gliomas. In some (but not all) of the larger gliomas, increased vascularity was associated with a 3 to 15 fold increase in K. Regional K values in malignant schwannomas were highly variable and generally could not be correlated to specific histologic features of the tumor except for some regions with increased vascularity.

Regional ^{14}C-misonidazole derived radioactivity (MISO*) was measured by quantitative autoradiography in experimental RT-9 brain tumors after IV administration. Misonidazole (MISO) concentration in plasma and brain was also measured by high pressure liquid chromotography. The non-metabolized fraction of ^{14}C-MISO*, fell gradually in plasma (0.89 at 4 hr) and more rapidly in brain (0.67 at 4 hr) and tumor (0.30 at 4 hr). MISO* distribution in tumor was variable and tumor concentrations relative to that in brain increased with time. The average tumor/brain MISO* ratio was 1.3, 1.7 and 2.6 at 0.5, 2 and 4 hours, respectivley, which suggests tumor uptake and binding of MISO, or more likely, MISO-derived ^{14}C-labeled metabolites. In addition, MISO* distribution in tumor tissue was strikingly heterogenous at 4 hours. Tumor regions with high MISO* activity correlated in part to viable-appearing cells around necrotic foci.

Regional ^{14}C-misonidazole derived radioactivity (MISO*) was measured by quantitative autoradiography in subcutaeous RT-9 experimental tumors after IV administration. Misonidazole (MISO) concentration in plasma, tumor, and other tissues was also measured by high pressure liquid chromatography. The distribution of MISO* in the tumors always resulted in a characteristic pattern with high peripheral and low central values. The limited distribution of MISO* to central tumor regions could be correlated to low values of blood flow and to diffusion from peripheral tumor regions. Low blood flow in the central regions of these tumors would significantly limit the distribution of drugs to viable-appearing cells in these areas and could account, in part, for the chemotherapeutic failures of certain solid tumors.

The apparent rate of glucose utilization (GU) in metastatic tumors was variable and not correlated with tumor size or location; high GU in medium and large-size tumors correlated with viable-appearing tissue and was always 1.3 to 3 times higher than that of adjacent and contralateral nontumorous brain. Local cerebral glucose utilization (LCGU) adjacent to small tumors was enhanced, whereas LCGU adjacent to large tumors was depressed. A general reduction of LCGU was induced by intracerebral metastatic tumors and correlated with tumor burden. The enhanced uptake of 2-deoxyglucose by viable tumor cells has diagnostic and localization value and suggests that appropriate glucose analogues could be developed to produce a tumor-selective inhibition of glycolysis.

A study of the distribution of dialysis fluid in the peritoneal cavity and the transport routes in the tissues surrounding the peritoneal cavity was undertaken in rats. Based on the distribution of the Evans Blue dye observed in the photographs of frozen sections, it is concluded that the dialysis fluid in a volume of 33 ml in a 200 g rat (2 liters when scaled to a human) distributes to essentially every surface in the peritoneal cavity. It appears that all tissues can potentially exchange with fluid in the peritoneal cavity. Absorption of

Evans Blue is not uniform in the wall of intestine, stomach, retroperitoneal muscle, and abdominal muscle. Heavy absorption of the dye was evident in parts of the abdominal wall and most of the diaphragm. These two locations showed very high concentrations of albumin (HSA) but lower concentrations in the case of EDTA (relative to HSA).

EDTA has high capillary permeability and moves into the plasma via the interstitial fluid and capillaries of the tissues surrounding the peritoneum. Large molecular weight substances such as HSA are, however, absorbed via the lymphatics. The diaphragm is thought to be the major site of lymphatic transport from the cavity and our HSA data for this tissue support this point. The intestine has a more developed lymphatic system than the abdominal wall, and greater transfer of HSA from the peritoneum into the plasma seems to occur via the lymphatics of the intestine than those of the abdominal wall.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM03506-20 LCHP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacology and Disposition of Antitumor Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Richard L. Cysyk, Head, Drug Metabolism Section, Lab. of Chem. Pharm., NCI

COOPERATING UNITS *(if any)*

Pediatric Oncology Branch, DCT, NCI
Clinical Pharmacology Branch, DCT, NCI

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Drug Metabolism Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A HPLC method was developed to quantitate SR-2508, a new hypoxic cell radiosensitizer, in the urine and blood of patients. Data have been collected on a single patient entered into a study at NIH who received 2.0 g/m2 IV. SR-2508 disappeared with a distribution halftime of 15 min and a plasma elimination halftime of 6.6 hr. The renal clearance of the drug was 106 ml/min and the renal excretion of the parent compound was 110% of the total dose administered. The metabolism of 6-mercaptopurine was studied in vitro and in patients. 6-Mercaptopurine ribonucleoside was found in the blood and urine of patients. Also, 6-mercaptopurine ribonucleoside was formed from 6-mercaptopurine by monkey liver microsomes.

Other Professional Personnel:

John M. Strong	Sr. Staff Fellow	LMCP, NCI
Sol Zimm	Medical Staff Fellow	PO, NCI
John Grygiel	Medical Staff Fellow	CP, NCI

Clinical Pharmacokinetic Study of SR-2508

A Phase 1 trial of the hypoxic cell radiosensitizer, SR-2508, was initiated by the Radiation Therapy Oncology Group (RTOG). In conjunction with this study we have developed a HPLC method to quantitate SR-2508 in plasma and urine. This analysis will be used to establish pharmacokinetic parameters in patients receiving SR-2508. To date, data collected on a single patient entered into the study at NIH who received 2.0 g/M^2 IV indicated a distribution halftime of 15 min and a plasma elimination half time of 6.6 hr. The renal clearance of the drug was 106 ml/min and renal excretion of the parent compound was 110% of the total dose administered.

Metabolism of 6-Mercaptopurine.

6-mercaptopurine ribonucleoside monophosphate is the most abundant metabolite of 6-mercatopurine observed in cell extracts. Also, 6-mercaptopurine ribonucleoside has been suggested as a possible metabolic product. We have identified 6-mercaptopurine ribonucleoside in urine and blood obtained from a patient who was receiving 6-mercaptopurine therapy. Evidence for the existence of 6-mercaptopurine ribonucleoside was obtained after separation of the compound by HPLC and mass spectral analysis of the collected material. An aliquot of the HPLC collection was also incubated with purine nucleoside phosphorylase and the resulting product, when analyzed by mass spectrometry, was identified as 6-mercaptopurine. When 6-mercaptopurine was incubated with monkey liver microsomes, 6-mercaptopurine ribonucleoside was detected. Additional studies are in progress to establish which enzymes present in the microsomal fraction are responsible for the production of this metabolite.

Publications:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM06108-14 LCHP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Action and Mechanism of Resistance of Antitumor Agents.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Richard L. Cysyk, Head, Drug Metabolism Section, Lab. of Chem. Pharm., NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Chemical Pharmacology

SECTION

Drug Metabolism Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	2.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The activation and covalent binding of Adriamycin to DNA and microsomal proteins was studied in isolated rat liver nuclei. Evidence was gained that VP-16 is metabolized by a peroxidative mechanism involving an oxygen-centered free radical. The substrate capacity of mAMSA and mAQDI for aldehyde oxidase was determined. A method developed to monitor the activity of thymidylate synthetase in intact cells was used to study the effects of MTX and fluoropyrimidines on the flux through this enzyme. The concentrations of 5-FU and MTX required to produce cell toxicity correlated with concentrations required to inhibit thymidylate synthetase in the intact cell.

Other Professional Personnel:

Paul E. Gormley	Medical Officer	LCHP
Birandra K. Sinha	Cancer Expert	LCHP
John M. Strong	Sr. Staff Fellow	LCHP

Free Radical Metabolism of VP-16 and Inhibition of Microsomal Lipid Peroxidation.
VP-16, an epipodophyllotoxin derivative, is an effective single agent for the treatment of small cell carcinoma of lung. VP-16 is also used in combination with other chemotherapeutic agents, adriamycin and cis-platinum, resulting in decreased toxicity. Adriamycin, a redox cycling drug, is known to generate reactive oxygen radicals which induce microsomal lipid peroxidation. Because of structural similarity of VP-16 with butylated hydroxytoluene (BHT), an antioxidant, it was of interest to examine whether VP-16 may inhibit lipid peroxidation. Incubation of daunomycin (100 μM) with liver microsomes in the presence of a NADPH generating system induced a four-fold increase in MDA formation. The addition of VP-16 to the incubation mixture inhibited peroxidation, which was dose-dependent. Studies suggest that this inhibition by VP-16 may be related to its ability to utilize H_2O_2 for activation since peroxidative metabolism of VP-16 produces an oxygen-centered free radical.

Binding of Adriamycin to Nuclear DNA.
Microsomal activation of adriamycin (ADR) in the presence of a NADPH generating system under anaerobic conditions produces reactive intermediate(s) which bind covalently to exogenously added nucleic acids and microsomal proteins. The nuclear membrane also contains reductase enzymes capable of activating ADR to its semiquinone free radicals. We have examined the activation and covalent binding of ADR to DNA in isolated nuclei. Incubation of ADR with rat liver nuclei under anaerobic conditions resulted in covalent binding of ADR to DNA. In contrast to microsomal proteins, however, less ADR is bound to nuclear DNA. The covalent binding of ADR is NADPH- and time-dependent. The presence of GSH and ethylxanthate decreases the binding. Since these agents also inhibit the covalent binding of ADR to microsomal proteins and exogenous DNA, this suggests that the mechanism leading to binding of ADR is similar between these two subcellular fractions.

Studies on the Mechanism of Action of mAMSA and Related Compounds
Studies on the mechanism of action of mAMSA were continued. The alkaline sucrose gradient technique for detecting DNA strand breaks was modified and is now more sensitive. The studies showing that inhibitors of microsomal enzymes do not protect against mAMSA induced DNA strand breaks (see last year's Annual Report) are being repeated using the more sensitive assay. Additional studies have been done on the inhibition of the enzyme aldehyde oxidase by mAMSA and its analogues. Inhibition of the enzyme by mAMSA is strictly competitive with a $K_i = 6 \times 10^{-8}$M. mAMSA has no observable effect on xanthine oxidase. The quinonediimine analogue of mAMSA, m-AQDI, is a non-competitive inhibitor of aldehyde oxidase. A more purified preparation of aldehyde oxidase is being attempted which will permit studies with a variety of electron acceptors to better define the site of inhibition of m-AQDI. Rabbit studies are planned to assess the effects of mAMSA inhibition of aldehyde oxidase in vivo. An HPLC assay has been adapted from the literature to measure MTX and 7-OH MTX in rabbit plasma. The

conversion of MTX to 7-OH MTX is through the aldehyde oxidase enzyme and the inhibition of this conversion will be used to monitor enzyme activity in vivo.

Studies on the effects of MTX and Fluoropyrimidines on Thymidylate Synthetase in Intact L1210 cells.

Attempts have been made this year to correlate results of MTX and fluoropyrimidine inhibition of TdR synthetase in intact L1210 cells with the toxicity of these drugs as determined by soft agar tissue culture. The concentrations of MTX and 5-FU required to produce toxicity in soft agar culture correlate well with the concentrations of drug required to inhibit TdR synthetase in the intact cell. Studies with a third drug, 5-FUdR, revealed a large disparity between the concentrations required to kill cells and to inhibit enzyme activity. TdR synthetase is inhibited at 10^{-9}M 5-FUdR while 10^{-3}M drug is nontoxic to cells in soft agar culture. This effect is thought to result from thymidine salvage by cells in the soft agar. Efforts are underway to grow L1210 cells in thymidine deficient soft agar to confirm this hypothesis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM06142-06 LCHP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Relationships Between In Vitro and In Vivo Drug Antitumor Action.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

D.S. Zaharko Head, Drug Kinetics & Therapeutics Section LCHP NCI

COOPERATING UNITS *(if any)*

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Laboratory of Chemical Pharmacology

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Drug Kinetics and Therapeutics

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	1.4	1.8

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of these experiments is to gain a better understanding of the mode of action of selected antitumor agents by conducting in vitro and in vivo studies with mouse tumor systems. L1210 leukemia and Lewis lung carcinoma were used to measure drug effects. In vitro studies, with hematoporphyrin and light cell damage on melphalan and actinomycin-D cytotoxicity, were concluded this year and indicated a synergistic response. 2'-deoxy-5-azacytidine (DAC) and some analogues have been studied in vitro and in vivo with L1210. Biochemical modulation occurs with thymidine and immune modulation occurs with pyran co-polymer. In vitro cytotoxicity studies with DAC indicate a maximum cell kill of 3 to 4 logs is achievable with optimal conditions of concentration and exposure time. In vivo cytotoxicity studies with DAC indicate log cell kills of greater than 7 logs at optimal doses. Reasons for this discrepancy are being studied. Advanced L1210 tumor in mice, with late treatment, indicate drug resistant cells (deoxycytidine kinase poor mutants) may be a major problem with DAC. Collaterally sensitive drugs such as cytoxan, BCNU, dihydro-5-aza-cytidine, 3-deazauridine and tiazofurin are being considered as potential solutions to this drug resistant.

Objectives

1. To relate the concentration and time of exposure of drugs and their cytoxic effects in vitro to the pharmacokinetics of drugs and their effects in vivo. 2. To explore the problem of drug resistance both in vivo and in vitro and to use means such as biochemical modulation, immune modulation and collaterally sensitive drugs in attempts to provide guide lines for the solution of this problem. 3. To elaborate our understanding of the L1210 model system as a means of comprehending drug action in vitro and in vivo, with respect to heterogeneity of growth rates and resistance development.

Methods and Major Findings:

1. Cytotoxicity of DAC on murine L1210 leukemia in vitro, was measured by soft agar colony formation. Concentrations of DAC were varied (0.001 to 100 µg(ml) and times of exposure were from 1 to 120 hrs. DAC was washed off cells before incubation of cells in the colony formation medium. Cytotoxicity is expressed as per cent of control viability at 10 and 15 days. The percent viability was first order in decay (linear on a log-linear plot of cytotoxicity vs dose) between 0.01 and 0.5 µg/ml of DAC and leveled off above 1.0 µg/ml for each exposure time. Exposure time of 24 hours and a concentration of 0.5 µg/ml gave the maximum cytotoxic response. Little additional cytotoxicity was caused by higher concentrations or longer exposures.

2. Antitumor action of DAC in vivo on advanced L1210 leukemia in CDF-1 mice indicates this drug is comparable to cytoxan and BCNU in cell killing ability in vivo following a single dose (>6-7 logs of L1210 kill following 1 x 10^5 cells i.p. of L1210 on day 0 and 50 mg/Kg of DAC either on day 3 or day 5). Development of resistance or indigenous resistance in L1210 precludes "cures" on late treatment with DAC.

Thymidine at high dose (~1 mM plasma concentration) although it modulates the toxicity of DAC (increased), it does not increase the therapeutic effect at equivalent toxic doses when given prior to or simultaneous with DAC.

Pyran copolymer, an immune modulator, appears to be able to eliminate the few remaining resistant cells to result in substantial "cures", after DAC treatment lowers the tumor burden to a few cells. If cytoxan is used to lower the tumor burden to a few cells, pyran copolymer does not result in substantial cures. Hence the combination DAC plus pyran appears worthy of further study.

3. Drug kinetic studies are being conducted using an HPLC reverse phase ODS column. Preliminary studies indicate a two phased plasma clearance of DAC with half lives of 1.7 min and 18 min following 100 mg/Kg of DAC i.v. in mice. In vitro half life of DAC in aqueous buffered media at pH 7.4 is 48-61 hrs at 25°C and 17-18 hrs at 37°C. Urinary clearance studies indicate that 50% of plasma elimination is accomplished via this route following a single i.v. dose.

Publications

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3. Zaharko, D.S. and Covey J.M. Modulation of Deoxycytidine Metabolism In Vivo with High Dose Thymidine, JNCI, in press, May 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CM06148-04 LCHP

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Endogenous Modifiers of Drug Action

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Richard L. Cysyk Head, Drug Metabolism Section LCHP NCI

COOPERATING UNITS *(if any)*
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SECTION
Drug Metabolism Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.0	4.5	2.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Stable isotope tracer methodology was used to monitor the flux through the de novo pyrimidine pathway in vivo. Recovery of pathway activity after PALA treatment in PALA-sensitive and PALA-resistant tumor lines was determined by quantitating the incorporation of stable-labeled carbon dioxide into uracil nucleotides by GC/MS. Stable-labeled ammonium chloride was found to be an effective precurser for pyrimidine biosynthesis in vivo. Also, in vitro studies demonstrated that ammonium chloride is utilized at the expense of glutamine as a precursor of carbamyl phosphate for the de novo synthesis of pyrimidines in isolated rat hepatocytes. Of twelve new compounds synthesized and tested as inhibitors of uridine kinase, one was found to be a potent inhibitor of uridine utilization by cultured L1210 cells. Riboxamide (NSC-286193) was also found to inhibit uridine utilization by L1210 cells at concentrations that are not growth inhibitory. The regulation of circulating purine concentrations by the isolated perfused rat liver was studied utilizing an artificial oxygen carrier and quantitating of purine uptake and export by HPLC.

Other Professional Personnel:

David Geffen	Medical Staff Fellow	LCHP
Jean M. Karle	Sr. Staff Fellow	LCHP
Louis Malspeis	Professor	Ohio State University
Patricia A. Monks	Visiting Associate	LCHP
John M. Strong	Sr. Staff Fellow	LCHP

Major Findings:

Use of Stable Isotope Tracer Methods to Monitor the Flux Through the De Novo Pyrimidine Pathway In Vivo.

An understanding of the spectrum of antitumor activity and the differential toxicity of inhibitors of de novo pyrimidine biosynthesis requires a knowledge of their effects on the flux through the de novo pyrimidine biosynthetic pathway in vivo, where all physiological factors pertaining to resistance or sensitivity are operative. As radioactive precursor incorporation into the total uracil nucleotide pool (Σuracil) of tissues in vivo is inadequate, we developed a GC/MS technique which enables measurement of ^{13}C incorporation from $^{13}CO_2$ into Σuracil of tumors in situ. CDF_1 mice with L1210 leukemia (sc) or Lewis Lung (LLCa) tumors were treated with 400 mg/kg PALA (ip) 1 to 48h prior to their exposure to 10% $^{13}CO_2$:90% O_2 for 20 min. Quantitation of Σuracil in the tumor was by HPLC and the $^{13}C/^{12}C$ ratio was measured by electron impact mass spectroscopy. The Σuracil pools of L1210 sc and LLCa tumors were similarly depressed from 4 to 24h after PALA treatment. LLCa tumors remained at depressed levels for 48h after the dose, while Σuracil in L1210 sc tumors had completely recovered to untreated levels. The incorporation of ^{13}C into the Σuracil pool of these tumors, as a measure of flux through the de novo pathway, was inhibited 1 and 4h after the dose of PALA in both tumor types and remained inhibited for 48h in LLCa tumors. However, in L1210 the rate of de novo synthesis was only 30-40% inhibited 12-24h after a dose of PALA, and had completely recovered its activity by 48h. This recovery of flux through the de novo pathway did not correlate with recovery of aspartate transcarbamylase activity. Thus, pathway flux rather than enzyme activity may be a determining factor in drug sensitivity. Current studies are examining the in vivo effects of other inhibitors of the de novo pathway.

In the Annual Report for last year, we described methodology to monitor the de novo pathway using ^{15}N-precursors (NH_4Cl or glutamine). The formation of di-labelled uracil (i.e. both nitrogens substituted) would allow us to calculate total product formed by the de novo pathway (i.e. both labelled and non-labelled) by using probability statistics as described in last year's Annual Report. We have been successful in making such determinations using isolated hepatocytes in vitro, and preliminary in vivo data indicate that such determinations may be possible for in vivo studies using a constant infusion of $^{15}NH_4Cl$ as the pathway precursor.

The Role of NH_4Cl and Glutamine as Substrates for De Novo Pyrimidine Biosynthesis In Vivo and In Vitro

The mammalian liver contains two separate carbamyl phosphate synthetase enzymes (CPS-I and CPS-II). CPS-II is a cytoplasmic enzyme which catalyzes the initial rate limiting reaction of de novo pyrimidine biosynthesis. CPS-I, which catalyses the initial step in the urea cycle, is found almost exclusively in the mitochondria of hepatocytes and is in greater abundance than CPS-II in these cells. The nitrogen donating substrates with the strongest affinity for these two enzymes are glutamine (CPS-II) and ammonia (CPS-I). However, ammonia has been reported to stimulate de novo pyrimidine biosynthesis in the liver as measured by increased $H\ ^{14}CO_3$ incorporation into uracil nucleotide pools (Σuracil). In an effort to elucidate the role of ammonia and glutamine as substrates for de novo biosynthesis of pyrimidines in the liver, we used the GC/MS technique described above to measure the incorporation of ^{15}N from these two precursors into Σuracil in isolated hepatocytes. Both glutamine and NH_4Cl stimulate the incorporation of $NaH^{14}CO_3$ into the uracil nucleotide pool of isolated hepatocytes. Following preincubation with varying concentrations of exogenous uridine, the incorporation was reduced up to 60% in the presence of glutamine, but only 4-6% in the presence of NH_4Cl. 5-^{15}N-Glutamine and $^{15}NH_4Cl$ were studied as precursors of de novo pyrimidine biosynthesis in hepatocytes utilizing a GC/MS technique to measure ^{15}N incorporation into the N_3 (from carbamyl phosphate [CP]) or N_1 (from aspartate) positions of the uracil moiety of nucleotides. In the absence of any competing substrate for de novo synthesis, 5-^{15}N-glutamine predominantly labeled the N_3 position of the uracil moiety (≈60% ^{15}N atom enrichment) but also contributed to the aspartate pool (≈20% N_1 atom enrichment). When $^{15}NH_4Cl$ was the precursor, it produced equal atom enrichment (≈90%) of the N_1 and N_3 positions, indicating virtual saturation of both CP and aspartate pools. In competition experiments cold NH_4Cl dramatiatically reduced the N_3 labeling of uracil by 5-^{15}N-glutamine, but barely affected N_1 labeling. In contrast, unlabeled glutamine caused marked stimulation of $^{15}NH_4Cl$ utilization, particularly for the N_3 position. Therefore, in our system NH_4Cl was utilized at the expense of glutamine as a precursor of carbamyl phosphate for the de novo synthesis of pyrimidines.

Regulation of Circulating Purine Concentrations by the Isolated Perfused Rat Liver. The liver is believed to be an important regulator of purine concentrations in plasma. To examine this regulation, isolated rat livers were perfused with the artificial oxygen carrier, Fluosol-43, and the export and uptake of purines determined by HPLC. The purines hypoxanthine, inosine, adenosine, and adenine were all found to be completely cleared in a single passage, but differed in their metabolic fate after uptake. Ninety-eight percent of hypoxanthine and inosine were catabolized to allantoin; 50% of adenosine was catabolized and 50% was phosphorylated; >99% of adenine was incorporated into the ATP pool. The liver effluent was 0.2-0.5 μM in xanthine, hypoxanthine, and inosine, and 0.5-0.7 μM in adenine; other purines (most notably, adenosine) were below detection. The export of purines was not influenced by incoming purines at physiological concentrations. Adenosine was released from the liver only under conditions of mild hypoxia. When the perfusion media was Krebs Ringer Bicarbonate, a challenge of 20 μM hypoxanthine caused sufficient hypoxia to induce the release of adenosine. Thus, the liver completely removes circulating purines and then exports hypoxanthine, inosine, xanthine, and adenine. Of these

exported purines, adenine appears to be the most likely candidate for purine salvaged by extra hepatic tissue. The subcellular source of adenine is not the adenine nucleotide pool and is presently under investigation.

Design, Synthesis, and Testing of Inhibitors of Uridine Kinase.
Previous studies in our Laboratory indicated that inhibition of pyrimidine salvage would enhance the activity of inhibitors of de novo pyrimidine biosynthesis. We have undertaken a new project to develop inhibitors of uridine kinase. To date, twelve compounds with modifications at the 5' position of uridine have been synthesized and evaluated. Eleven of the compounds were of no greater potency than compounds previously studied by others. One of the compounds (a 5'-nitropyrimidine) was found to completely block the phosphorylation of uridine by intact L1210 cells at inhibitor concentrations of 50 μM. This compound will be studied in combination with inhibitors of de novo pyrimidine biosynthesis.

Effect of Riboxamide (NSC-286193) on Pyrimidine Metabolism
Riboxamide is a potent inhibitor of purine biosynthesis. High concentrations of Riboxamide had no effect on the export of purine by the isolated perfused rat liver. There was, however, a dramatic change in the metabolism of circulating uridine. The half-life of a radioactive spike of uridine was increased from 7 min to 75 min; the clearance of hyperphysiologic concentrations of uridine was decreased; and the concentration of circulating uridine was increased from control levels of 1-2 μM to 7.5 μM. Riboxamide was found to be a potent inhibitor of uridine uptake and/or phosphorylation by cultured L1210 cells at concentrations that do not inhibit cell growth. Thus, Riboxamide might have some importance as an inhibitor of pyrimidine salvage.

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ANNUAL REPORT OF THE LABORATORY OF EXPERIMENTAL THERAPEUTICS AND METABOLISM

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

Program Objectives:

The Division of Cancer Treatment (DCT) has the charge to develop new and improved modalities of cancer treatment. The objective of the Developmental Therapeutics Program (DTP) within DCT is to discover or develop new chemotherapeutic agents to be used alone or in combination therapy. As a part of the DTP, the goal of the Laboratory of Experimental Therapeutics and Metabolism (LETM) is to generate basic information which will lead to new insights into tumor biology, biochemistry, pharmacology and toxicology that will contribute to the improvement of cancer treatment.

Specific research project areas of the LETM were described in detail in the FY 1982 Annual Report and are not repeated here. Current research progress for FY 1983 is summarized below.

PROJECT REPORTS:

Isolation and Purification of Major Lung Cell Types. Techniques for isolating and characterizing the major cell-types of lungs from a number of animal species are being developed. From rabbit lung, both alveolar type II and bronchiolar Clara cells have been isolated. These cell-types are presently being used to investigate the pulmonary cytochrome P-450 system using acetylaminofluorene as substrate. Considerable work into isolating rat lung endothelial cells has established a number of enzymatic markers that are being used to identify this cell-type.

Paraquat/NADPH Interactions. Chemically-reduced paraquat forms at least two distinct adducts in the presence of NADPH or NADH. This reaction was sensitive to the presence of oxygen. In both rat liver and lung subcellular fractions, no interaction between paraquat and the pyridine dinucleotides was apparent under either aerobic or anaerobic conditions. Presently, the possible formation of adducts from paraquat *in vivo* is being investigated and the possible importance to alteration of nucleotide ratios and toxicity to the lungs is being examined.

Effect of Oxygen on the Metabolism of Nitrofurantoin in Perfused Rat Lung. This study explored whether intact lung tissue could reductively metabolize the lung-toxic antibacterial drug, nitrofurantoin (NF), in a similar manner as pulmonary microsomes and if the resulting metabolism was modified by oxygen. At least two metabolites of NF were evident in perfused lungs after 1 hr exposure. Further, considerable binding to tissue macromolecules was also measured. Both metabolism and covalent binding were inhibited by oxygen.

These results were observed similarly in rat lung homogenates. Experiments are presently under way to identify the nitrofurantoin-derived metabolic products, and to determie if these data may help explain the oxygen enhancement of NF lung toxicity.

Pharmacokinetics of Doxorubicin in Isolated Perfused Lung of Dog and Man. Doxorubicin was concentrated in isolated dog lung during a 50 min perfusion in situ. The uptake of drug increased with time but was saturable at perfusate concentrations exceeding 20 nmol/ml. Changes in the kinetic model parameters suggested that, at high perfusate concentrations, doxorubicin influx was inhibited. These studies have lead to a Phase I clinical trial utilizing the hemiperfusion technique in metastatic sarcoma patients. The surgical and perfusion procedures were successfully performed in all the patients examined to date. However, in human lung, doxorubicin accumulation was considerably slower than in the dogs. The concentration of drug in biopsied tumors from the patients was consistently less than the surrounding tissue.

Biochemical and Physiological Effects of Lung Perfusion with Doxorubicin In Vivo. A dose-related morbidity was seen in dogs following isolated in situ right lung perfusion. Lactate dehydrogenase activity in the perfusate increased as dose was increased, indicating tissue damage during the perfusion. Up to 7 days post-perfusion, marked changes were seen in the serum protein concentrations although these were independent of doxorubicin concentrations. Serum lactate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, showed a dose-dependent increase 2 hrs and 1 day after the lung perfusion. Plasma angiotensin converting enzyme activities up to 14 days post-perfusion depended upon the concentrations of doxorubicin used during the perfusion. The study has demonstrated that doxorubicin produces dose-dependent damage to the pulmonary tissue. However, the observed injury only appeared life-threatening at perfusate drug concentrations in excess of 20 nmol/ml. In situ lung perfusion for the treatment of unresectable pulmonary tumors may therefore be clinically applicable.

Enzyme Kinetics of Acetylaminofluorene Metabolism. At least 2 forms of hepatic cytochrome P-450 were responsible for the formation of the 1, 3, 5, 7 and 9-OH derivatives of the carcinogen, acetylaminofluorene, while N-hydroxylation appeared to only involve one distinguishable isoenzyme. The activation of acetylaminofluorene (N-hydroxylation) was induced by TCDD whereas only 7-hydroxylation was induced by phenobarbital.

Localized Production of Reduced Oxygen Species in the Lung. A cerium precipitation method is being developed to investigate localized production of superoxide in lung. Extensive studies using Ce/H_2O_2 in the presence of a range of antioxidant enzymes and superoxide traps were undertaken in order to characterize the interaction of cerium with reduced oxygen. The rate of this reaction was biphasic, pH-dependent and inhibited by ascorbic acid, superoxide dismutase and albumin but not by ethanol or mannitol. In lung slices from rats treated with the lung-toxic drug, nitrofurantoin (a compound which enhances superoxide production), cerium-derived electron dense bodies were seen principally around the alveolar type II cells. These bodies were diminished in the presence of catalase but markedly increased by superoxide dismutase.

Studies are presently under way to quantify the precipitated cerium under various conditions using X-ray analysis and labeled cerium.

Nephrotoxicity of Nitrosoureas. A reliable model of MeCCNU renal damage in BDF mice and Fischer 344 rats has been developed. Histopathological changes produced by the drug were closely paralleled by marked changes in biochemical parameters (e.g., PAH transport) measurable in vitro in kidney slices, as well as by certain in vivo renal function tests (e.g., urinary osmolality and excretion of kidney-derived urinary enzymes). Preliminary studies show the Fischer rat to provide a relevant model also for studying the nephrotoxicity of other nitrosoureas. For example, streptozotocin was found to be acutely nephrotoxic, as measured by in vivo renal function tests, whereas, low doses of chlorozotocin result in a chronic progressive nephropathy.

Distribution, Metabolism and Covalent Binding of Methyl-CCNU in Rats. Studies were conducted in Fischer rats administered MeCCNU labeled either within the carbamoylating [(cyclohexyl-1-^{14}C)-MeCCNU] or alkylating [(2-chloroethyl-1,2-^{14}C)-MeCCNU] region of the compound. Body fat accumulated the highest tissue levels (nmoles/g wet weight) of parent compound (determined by HPLC analysis of tissue extracts). Kidney accumulated the highest tissue levels of the more polar ether and methanol extractable metabolites and/or degradative products of MeCCNU. Moreover, carbamoylation of tissue protein by (cyclohexyl-1-^{14}C)-MeCCNU and alkylation of DNA by (2-chloroethyl-1,2-^{14}C)-MeCCNU were higher in kidney than in any other tissue. Thus, as yet, no clear-cut case can be made for either carbamoylation or aklylation for MeCCNU-induced nephropathy.

Effect of BCNU on Pulmonary and Serum Angiotensin Converting Enzyme. In vitro, BCNU had a direct inhibitory effect on both serum and pulmonary ACE of rats, that was dependent both on time and the concentration of BCNU. In vivo, a large single dose (80 mg/kg, i.p.) did not alter pulmonary ACE nor cause histologically observable acute lung damage. However, serum ACE dropped by 25% within 1 hour after BCNU. A multi-dose regimen, consisting of 5 mg BCNU/kg once per week for 6 weeks, caused marked pulmonary injury, which continued to develop in severity over several weeks following the completion of dosing. Lung ACE after 4 doses (total of 20 mg BCNU/kg) was depressed by 40% and remained low until dosing was completed. Following the final dose, lung ACE returned to control within 2 weeks. However, after an additional 2 weeks, both lung and serum ACE had decreased by 35% and 25%, respectively and remained low for the duration of the study. ACE may provide a useful biochemical monitor for BCNU-induced pulmonary toxicity, but careful attention must be given to the time course of changes in the enzyme activity.

Effect of BCNU on Pulmonary Glutathione Reductase. Single doses of 8 mg BCNU/kg or 80 mg BCNU/kg inhibited lung GSSG reductase by 11% and 82%, respectively. The inhibition was very persistent, lasting up to 7 days after a single dose of BCNU. The multi-dosing regimen of 5 mg BCNU/kg/week for 6 weeks results in the development of severe pulmonary damage. After 3 weeks of BCNU dosing, GSSG reductase activity decreased to 50% of control values and GSSG levels rose to 227% of control values. After 6 weeks of BCNU, GSSG reductase activity decreased to 22% of control values. BCNU also inhibited pulmonary GSSG reductase in vitro. The inhibition was dependent on length of incubation time and the concentration of BCNU. Other nitrosoureas, methyl-CCNU and CCNU, also

inhibited GSSG reductase while streptozotocin and chlorozotocin had little if any effect. The in vitro inhibition can be mimicked by N-ethylmaleimide which binds to free sulfhydryl groups. Addition of sulfhydryl donors, such as GSH and dithiothreitol, into the incubation media prevents the inhibition of GSSG reductase by BCNU. The glutathione redox system is part of an important antioxidant defense mechanism in pulmonary cells and further studies are under way to explore whether impairment of this system by BCNU ultimately leads to or contributes to pulmonary toxicity.

Isolation and Characterization of Reactive Metabolites of Methylfurans. Acetylacrolein (AA), a product of peracid oxidation of 2-methylfuran (2-MF), a naturally occuring cytotoxic furan, was investigated as a possible microsomal metabolite of 2-MF. Glutathione, N-acetylcysteine and cysteine adducts of AA although apparently rapidly formed, were not stable enough to allow isolation. When rat liver microsomal incubations were performed with semicarbazide (SC), NADPH and 2-MF, a metabolite was isolated by gel permeation chromatography and HPLC and was found to be identical by mass spectroscopy to the disemicarbazone of AA. Methylbutenedial, the analogous oxidative ring opened product from 3-MF was isolated as the disemicarbazone from rat liver microsomal incubation with 3-MF. Addition of SC to rat lung microsomes containing ^{3}H-3-MF, and NADPH significantly inhibited covalent binding of ^{3}H-3-MF metabolites to microsomal proteins, although a considerably greater amount of 3-MF was metabolized in the presence of SC. Coordinate with the decreased covalent binding there was the appearance of the radiolabeled semicarbazide conjugate of methylbutendial. Thus, the dialdehyde appears to be the reactive metabolite of 3-MF that is capable of binding covalently to tissue macromolecules. Moreover, since the covalent binding of reactive material is directly correlated with the occurrence of toxic lesions, the dialdehyde is probably the ultimate toxic metabolite involved.

Improved Assay Methodology for Hydroxyfatty Acids from Lipid Peroxidation. Hydroxyfatty acids were released from phospholipids by enzymatic hydrolysis. The acids were then separated by an HPLC procedure using a reverse-phase chromatography system and were detected and quantitated, against synthesized standards, by UV absorption at 235 nm. This procedure was used successfully to demonstrate the presence of 15-hydroxyeicosatetraenoic acid and 9- and 13-hydroxyoctadecadienoic acids in livers from mice treated in vivo with necrogenic doses of carbon tetrachloride. Livers and lungs from rats given carbon tetrachloride, or from mice given paraquat, showed HPLC peaks indicative of hydroxyfatty acids, although none corresponded exactly with the synthetic standards available. Further studies will continue to probe the development and potential applicability of this methodology to the investigation of the role of lipid peroxidation in tissue injury.

Immunological Characteristics of Beige-Nude Mice; Utility for Cancer Research. We have investigated the effects on several cellular immune functions of the transfer of nude genes to beige mice. The resulting viable, double homozygous recessive bg/bg nu/nu (beige-nude) mice combine the relative absence of T-cell function in regular nude mice, known to have high NK levels with the very low NK activity of beige mice. Therefore, such double immune deficient mice provide new possibilities for studying immune surveillance, particularly for establishing to what extent NK cells take part in host defense against spontaneous,

induced, or transplantable tumors. Interestingly, no spontaneous malignant tumors have so far been observed in beige-nude mice.

Pathology of BCNU-Induced Lung Injury. Histopathological and electron microscopic studies of lung lesions induced in F344 rats by chronic BCNU treatment showed that the animals developed a severe interstitial fibrosis, emphysema, atelectasis, chronic bronchitis and peribronchitis as well as pneumonia. Macroscopically visible nodular lesions were identified as proliferated serous cells of peribronchial submucous glands accompanied by fibrosis and inflammation. Preneoplastic lesions (bronchial and bronchiolar hyperplasia, early squamous metaplasia, adenoma of peribronchial submucous gland and squamous metaplasia of proliferated submucous gland) were also found.

Pathology of Doxorubicin-Perfused Lungs of Dogs. The histopathology of dog lungs perfused in situ with different concentrations of doxorubicin was studied. The acute effects were severe perivascular and subpleural edema and emphysema. Up to 4 weeks after the start of treatment, pneumonia, pleuritis, atelectasis and early interstitial fibrosis developed from such lesions.

Collaborative Electron Microscopy Studies. A. Improved methods to produce consistent preparations of liposome-entrapped Melphalan were developed; B. The electron microscope was utilized to show that many nucleotide analogs of GDP and GTP supported polymerization of tubulin; C. Ultrastructural characterization of malignant human cell lines maintained in vivo in athymic host systems showed that various melanomas sublines had several distinctive morphological features, including intracytoplasmic filaments and electron-dense deposits within the cytoplasm.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07131-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and purification of major lung cell types

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Rodney F. Minchin, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Many pulmonary toxins exert discrete and localized reactions within the lungs suggesting that the heterogeneous population of lung cells vary considerably with respect to interactions with xenobiotics. In order to elucidate the differential effects of various toxins on the lung cell populations, techniques for isolating and characterizing the major cell-types from a number of animal species are being developed. The cell-types of principal interest include the vascular endothelial cells, alveolar type I and type II cells, and the interstitial fibroblasts and pericytes which constitute over 90% of the lung mass. Further, the bronchiolar Clara cells and alveolar macrophages are also of interest because of their known metabolic activities. Cells are characterized after dispersing the lung tissue into a single cell suspension with mild collagenase treatment. They are separated principally by centrifical elutriation using microprocessor control flow and/or density gradients. This technique permits the separation of heterogeneous cell populations not only according to size but also according to density. Cell-types are identified using specific histochemical and biochemical markers. Parallel studies into the characteristic morphology of the major cell-types are under way utilizing electron microscopy. From rabbit lung, both alveolar type II and bronchiolar Clara cells have been isolated. These cell-types are presently being used to quantify the distribution of the pulmonary cytochrome P-450 system using acetylaminofluorene as substrate. Considerable work into isolating rat lung endothelial cells has established a number of enzymatic markers that are being used to isolate and purify this cell-type. The endothelial cells will be used to study the effects of various toxins on the integrity and function of these cells.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Peter Ho, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

Pulmonary metabolic systems are capable of activating and detoxifying a range of potentially toxic compounds as well as modifying the kinetics of many circulating endogenous and exogenous substrates. These non-respiratory functions of lung appear to be localized within specific cell-types although investigations in this area have been limited by the heterogeneous nature of lung tissue. A number of recent studies have been aimed at isolating and identifying various cell-types, in particular the alveolar type II and nonciliated bronchiolar epithelial (Clara) cells. However, most established procedures are limited by the low yield of cells, a complex and time-consuming isolation procedure, or a poor final purity of the cell population. The objectives of this study are to develop improved methods for the isolation of defined lung cell populations which will allow the investigation of the biochemical mechanisms of pulmonary toxins at a cellular level. The principal cells of interest include the capillary endothelial cells, alveolar type I and II cells, and the interstitial fibroblasts and pericytes. These cell-types constitute over 90% of the cell mass. Further, the bronchiolar Clara cell is of major interest because of its known cytochrome P-450 mixed function oxidase activity.

Methods Employed:

Initial studies were undertaken in rabbit lung where the type II and Clara cells were successfully isolated and identified using published techniques with some modification. The use of strong proteolytic agents to disperse the lung tissue causes irreversible damage to many cell-types and compromises their metabolic activity. Further, since the identification of some cell-types such as the capillary endothelial cell depends upon specific surface proteins, general proteases have limited use in producing single cell suspensions of lung tissue. As a consequence, a method using mild collagenase treatment was developed which results in a high cell yield with greater than 95% viability. The cell types are separated using centrifugal elutriation.

The methods of cell identification being used or anticipated to be used include: 1) Endothelial cells: primary amine fluorescence, 5-hydroxytryptamine metabolism, angiotensin converting enzyme activity, monoamine oxidase histochemistry; 2) Type II cells: modified papaniculou stain, neutral red fluorescence, phospholipid synthesis, alkaline phosphatase activity; 3) Clara cells: 4-ipomeanol covalent binding, nitroblue tetrazolium staining; 4) Macrophages: phagocytosis, superoxide production following phorbol ester stimulation; 5) Polymorphonuclear leukocytes: superoxide production following phorbol

ester stimulation, Wright stain; 6) Fibroblasts: collagen synthesis, colony proliferation, collagen antibody interaction.

Major Findings:

Studies are presently under way in collaboration with the Laboratory of Chemical Carcinogenesis, DCPT, to characterize the metabolism of 3-acetylaminofluorene in rabbit lung microsomes, type II and Clara cells. Earlier collaborative investigations have shown that the metabolism of 3-acetylaminofluorence in liver involves a number of cytochrome P-450 isoenzymes and therefore this substrate can be used to quantify heterogeneous distributions of cytochrome P-450 populations. The monooxygenase activity in lung appears to be confined to selective lung cells such as the bronchiolar Clara and alveolar type II cell although good investigative probes for cytochrome P-450 activity in lung cells are lacking. The sensitivity of the 3-acetylaminofluorene assays permits this carcinogen to be used to study cytochrome P-450 activity in lung as well.

At present, another major emphasis of this project centers on the purification of rat lung endothelial cells which will be used to study the effects of various toxins such as BCNU, paraquat, monocrotaline and α-naphthylthiourea on endothelial cell function. Highly sensitive radiometric assays for angiotensin converting enzyme and monoamine oxidase activity which can be used with a limited quantity of cells have been established. Further, 5-hydroxytryptamine fluorescence and monoamine oxidase histochemistry have been established as procedures for identitying and quantifying the purity of the endothelial cell fractions.

Significance to Biomedical Research and the Program of the Institute:

The development of new or improved procedures for lung cell isolation is essential to permit the more detailed exploration of pulmonary metabolism and physiological/biochemical functions as well as mechanisms of toxicity/carcinogenesis involving the lung. This is also an important step in the direction of more rational development of better drugs for lung cancer treatment, based on increased knowledge of normal lung cell populations that may be susceptible to neoplastic transformation.

Proposed Course:

Future studies will attempt to isolate and characterize the other major lung cell-types for use in studies aimed at defining the localized initiation of cellular damage by known lung toxins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07132-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Paraquat/NADPH interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Rodney F. Minchin, Visiting Fellow.
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several studies have shown that the pulmonary toxin, paraquat, produces a marked decrease in lung NADPH without a concomitant increase in $NADP^+$. These results suggest that paraquat either inhibits the synthesis of the pyridine dinucleotide or enhances its degradation. To investigate the possible mechanism and consequences of these observations, studies were undertaken to: 1) examine the direct interaction of paraquat with NADPH, 2) study the effect of paraquat on NAD/NADP synthesis and 3) determine if paraquat increases pyridine dinucleotide degradation by enhanced synthesis of poly ADP-ribose. Paraquat interaction with several pyridine dinucleotides was investigated after both chemical and enzymatic reduction of the substrates. Reaction products were analyzed by high pressure liquid chromatography. The intereaction of paraquat with pyridine dinucleotide was also studied in rat liver and lung microsomes, lung 9000 g supernatant and after intraperitoneal injection of several doses *in vivo*. Chemically-reduced paraquat forms at least two distinct products in the presence of NADPH or NADH. This reaction was sensitive to the presence of oxygen. In both rat liver and lung subcellular fractions, no interaction between paraquat and the pyridine dinucleotides was apparent under either aerobic or anaerobic conditions. Presently, the possible formation of similar products from paraquat *in vivo* is being investigated. Subsequent experiments will quantify the effects of paraquat on the synthesis and degradation (e.g., formation of poly ADP-ribose) of NAD^+ and $NADP^+$ in order to determine if such mechanisms may account, in part, for the depletion of pulmonary nucleotides following paraquat administration.

Objectives:

Paraquat is a commonly used herbicide with reported pulmonary toxicity in both animals and man. Several laboratories, including our own, have observed a decrease in lung pyridine dinucleotide content following paraquat treatment in rats. These observations cannot be explained by simple oxidation of the dinucleotide cofactors as has been previously suggested. The data indicate that paraquat either inhibits the synthesis of the pyridine dinucleotides or enhances their degradation. A critical equilibrium usually exists between NAD^+, $NADP^+$, ATP and the enzymatic activity of a number of essential pathways such as the pentose shunt, mitochondrial respiration, cyclic nucleotide synthesis and protein synthesis. Depletion of the pyridine dinucleotides may represent a biological response to paraquat that is detrimental to the cellular system leading to eventual toxicity. The present study was undertaken to investigate this hypothesis. The principal objectives are to: a) study any direct interaction between paraquat and the pyridine dinucleotides, b) investigate the synthesis of NAD^+ and $NADP^+$ in the presence and absence of paraquat, c) study any possible enhancement in the degradation of the dinucleotide cofactors by paraquat, d) correlate any observed changes with the onset of paraquat lung injury *in vivo*.

Methods Employed:

Chemically reduced paraquat was produced by the addition of excess sodium dithionite. Resulting products were analyzed by reverse phase high pressure liquid chromatography. Rat liver and lung subcellular fractions were obtained by differential centrifugation and anaerobic conditions were obtained either by purging all solutions with nitrogen or by the use of a glucose/glucose oxidase/catalase system.

Major Findings:

Chemically reduced paraquat formed at least two distinct products in the presence of NADH or NADPH but not with either NAD^+ or $NADP^+$. The observed reaction was oxygen sensitive and although neither of the products have yet been identified, the less polar product chromatographed with a similar retention time to that of N-methylnicotinamide.

Under aerobic conditions, reduction of paraquat with either rat liver or lung microsomes did not produce paraquat-derived products as previously observed following chemical reduction. Similar results were observed using rat lung 9000 g supernatants. Furthermore, following the purging of oxygen from the lung preparations with either nitrogen or with a glucose/glucose oxidase/catalase system in order to obtain anaerobic conditions, no metabolic product of paraquat were apparent.

Following the administration of ^{14}C-paraquat to rats, the pulmonary radioactivity was analyzed by HPLC. Inconclusive results have been obtained because of the variability in the *in vivo* data. Studies are under way to examine alternative mechanisms for recovering and concentrating paraquat-derived radiolabel following *in vivo* administration. Results to date indicate that, if

paraquat directly interacts with the pyridine dinucleotides, such a reaction must take place essentially under reduced oxygen tensions and at maximal reducing capacity.

Significance to Biomedical Research and the Program of the Institute:

Further elucidation of biochemical features associated with paraquat toxicity will lead to a better understanding of its adverse effects on the lung. Fundamental mechanistic information on this model lung toxin may have immediate implications for other lung toxic chemicals, including some drugs used in cancer chemotherapy.

Proposed Course:

Future studies in this project will involve investigating the synthesis of $NAD^+/NADP^+$ in intact lung tissue. Since this synthesis pathway is reportedly sensitive to superoxide, a significant reduction in quinolate phosphoribosyl transferase, a critical enzyme in the NAD^+ synthesis scheme, may account for the observed alterations in the pyridine dinucleotide levels in lung following paraquat treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07133-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of oxygen on the metabolism of nitrofurantoin in perfused rat lung

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Rodney F. Minchin, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Nitrofurantoin is an antibacterial agent with numerous reported occurrences of pulmonary injury in animals and man. Previous studies indicated that nitrofurantoin is enzymatically reduced in lung microsomal preparations to an unstable reactive anion which has been implicated as a possible cause of the nitrofurantoin induced lung toxicity in vivo. The anion radical is thought to reduce oxygen to superoxide which can subsequently react with cellular constituents. The present study was undertaken to examine if intact lung tissue could reductively metabolize nitrofurantoin in a similar manner as pulmonary microsomes and if the resulting metabolism was modified by oxygen.

Experiments were performed using a recirculating in situ perfused rat lung procedure. A specific and sensitive high pressure liquid chromatographic assay was established to analyze the resulting nitrofurantoin metabolites. Parallel studies were undertaken in lung homogenates in order compare the metabolism of nitrofurantoin in homogenates to that obtained in intact lung tissue. Fixed perfused lung tissue has also been examined by light and electron microscopy in order to evaluate the effects of the experimental procedure on the integrity of the lung.

At least two metabolites of nitrofurantoin were evident in perfused lungs after 1 hr exposure. Further, considerable binding to tissue macromolecules was also measured. Both metabolism and covalent binding were inhibited by oxygen. Similar results were observed in rat lung homogenates. Experiments are presently under way to identify the nitrofurantoin-derived metabolic products.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Viji Ravindranath, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Anthony A. del Campo, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

Nitrofurantoin (NF) is an antibacterial agent used primarily to treat urinary tract infection and has been shown to induce pulmonary damage in both animals and man. Previous studies from our laboratory have shown that in the presence of lung microsomes or cytosol, NF can undergo one-electron reduction to form an unstable anion capable of reducing molecular oxygen to superoxide. Under anaerobic conditions, the reduced NF moiety is susceptible to further reduction resulting in an intermediate(s) capable of covalently binding with cellular macromolecules. Thus the redox cycling of nitrofurantoin with molecular oxygen, and its further reduction to alkylating intermediates, represent competitive pathways.

Because of the in vitro observation regarding nitrofurantoin metabolism, it has been postulated that the redox cycling of this drug and the subsequent formation of toxic quantities of superoxide may represent the mechanism of nitrofurantoin pulmonary damage.

The objectives of the present study were to establish whether nitrofurantoin undergoes reductive metabolism in intact lung tissue, to identify the metabolic products and to eventually examine if the metabolism of nitrofurantoin is related to or correlated with the drug's toxicity.

Methods Employed:

^{14}C-Nitrofurantoin metabolism and covalent binding were measured using an isolated perfused rat lung preparation and rat lung 9000 g homogenates. Radiochromatograms were produced with a specific high pressure liquid chromatographic assay. Some lung tissue was fixed in 3% glutaraldehyde and prepared for electron microscopy. Methods to be used to identify the metabolites of nitrofurantoin will involve both nuclear magnetic resonance and mass spectrometry.

Major Findings:

Two separate products were apparent by high pressure liquid chromatography. The quantity of metabolites produced was inversely related to the oxygen tension used to respire the lungs. The extent of the covalent binding of nitrofurantoin-derived radioactivity in lung was also enhanced under anaerobic conditions although significant covalent binding was still evident at high oxygen tensions.

The results support previous in vitro findings that indicated that oxygen inhibited the formation of stable reduced products of nitrofurantoin. Furthermore, intact tissue is capable of reducing nitrofurantoin to reactive intermediates. A similar metabolic profile was seen in rat lung 9000 g homogenates. The enhanced formation of metabolites in both homogenates and perfused lung under anaerobic conditions is indicative of reductive metabolism of nitrofurantoin. Studies are presently under way to isolate and identify the major metabolites using mass spectroscopy and nuclear magnetic resonance.

Significance to Biomedical Research and the Program of the Institute:

Further elucidation of the nitrofurantoin metabolism and the reductive activation of molecular oxygen in intact pulmonary tissue will result in a better understanding of a lung toxicity mechanism that may be common to many different chemical compounds, including some drugs used in cancer chemotherapy.

Proposed Course:

The proposed future course of this project is to attempt to correlate biochemical changes such as lipid peroxidation, fatty acid synthesis, pentose shunt and other enzymatic processes with the metabolism of nitrofurantoin under conditions that either enhance or inhibit toxicity as determined in whole animal models.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07134-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical and physiological effects of lung perfusion with doxorubicin in vivo

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Rodney F. Minchin, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The biochemical and physiological effects of in situ isolated lung perfusion was investigated in dogs using the anticancer agent, doxorubicin. During perfusion and for the first 2 weeks post perfusion, biological samples were collected for various enzymatic analyses in order to elucidate possible correlations between administered drug dosage and subsequent toxicity. Two weeks post perfusion, a right-lung pneumonectomy was performed on surviving dogs in order to assess the functional capacity of the perfused left lungs. A dose-related morbidity was seen in the animals following lung perfusion. Lactate dehydrogenase activity in the perfusate increased as dose was increased indicating tissue damage during the perfusion. Up to 7 days post-perfusion, marked changes were seen in the serum protein concentrations although these were independent of doxorubicin concentrations. Serum lactate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, showed a dose-dependent increase 2 hrs and 1 day after the lung perfusion. Plasma angiotensin converting enzyme activities up to 14 days post-perfusion depended upon the concentrations of doxorubicin used during the perfusion. The study has demonstrated that doxorubicin produces dose-dependent damage to the pulmonary tissue. However, the observed injury only appeared life-threatening at perfusate drug concentrations in excess of 20 nmol/ml. In situ lung perfusion for the treatment of unresectable pulmonary tumors may be clinically applicable.

Other Professional Personnel:

Michael R. Johnston, Surgery Branch, NCI, Bethesda, Maryland

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Michael A. Aiken, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Richard Terrill, Surgery Branch, NCI, Bethesda, Maryland

Objectives:

Previous studies from our laboratory have explored the feasibility of in situ isolated lung perfusion with doxorubicin as a means of treating metastatic sarcoma localized in pulmonary tissue. These investigations have shown that, kinetically, the procedure may have certain advantages over systemic drug administration. In rat and dog lung, doxorubicin was found to be taken up slowly from the vasculature but to an extent where lung tissue concentrations exceeded perfusion concentrations. Initial studies in man have indicated that human lung resembles that of dog with respect to doxorubicin uptake and retention although the rate of drug accumulation was less.

The potential usefulness of in situ lung perfusion for the treatment of various lung cancers may be determined by both the extent of drug uptake and the extent of lung toxicity resulting from the procedure. As previously reported, DOX uptake in dog lung was dependent both upon drug concentration and time, reaching maximum tissue levels at perfusate concentrations greater than 20 nmol/ml of blood. Further, disposition in lung was found to be well described by a simple diffusion model allowing an estimation of drug uptake with time.

The purpose of the present study was to investigate the biochemical and physiological changes induced by perfusion of dog lung in situ with DOX. The relationship between these changes and perfusion drug concentration, and their possible usefulness in predicting or assessing tissue damage, were considered in detail.

Methods Employed:

An isolated in situ perfusion of the left lung was performed in each dog. Briefly, the left lung lobes were perfused via the left pulmonary artery with whole dog blood maintained at 37°C, and pH 7.3-7.5. The venous return from the perfused lung was isolated, cannulated and drained into a cardiotomy reservoir. The tissue was perfused for 50 min at a flow of 300 ml/min. Pulmonary biopsies, systemic blood and reservoir blood samples were collected at appropriate intervals for drug and enzyme assays. Following perfusion, the lungs were flushed with 200 ml of systemic blood by loosening the tourniquet around the arterial cannula. All cannulae were then removed and the chest was closed. Two weeks

post-perfusion, a right pneumonectomy was performed in order to assess the functional capacity of the left lung which had undergone perfusion. Surviving dogs were sacrificed two weeks after the pneumonectomy and autopsied.

Major Findings:

A dose-related morbidity was seen in the animals following lung perfusion. Lactate dehydrogenase activity in the perfusate increased as dose was increased indicating tissue damage during the perfusion. Up to 7 days post-perfusion, marked changes were seen in the serum protein concentrations although these were independent of doxorubicin concentrations. Serum lactate dehydrogenase, glutamate oxaloacetate transaminase and glutamate pyruvate transaminase, showed a dose-dependent increase 2 hrs and 1 day after the lung perfusion. Plasma angiotensin converting enzyme activities up to 14 days post-perfusion depended upon the concentrations of doxorubicin used during the perfusion. The study has demonstrated that doxorubicin produces dose-dependent damage to the pulmonary tissue. However, the observed injury only appeared life-threatening at perfusate drug concentrations in excess of 20 nmol/ml. *In situ* lung perfusion for the treatment of unresectable pulmonary tumors may be clinically applicable.

Significance to Biomedical Research and the Program of the Institute:

This project is designed to define a new and better approach to treat unresectable tumors in the lungs. In addition, the methodology and the approaches taken will yield much new basic scientific information concerning pulmonary pathophysiology.

Proposed Course:

Studies are presently under way to establish a complete lung perfusion technique in dogs that can be used to examine the acute and chronic effects of doxorubicin and other anticancer agents in the lungs.

While the results of the present study clearly establish a dose-related toxicity of doxorubicin during lung perfusion, it is still unknown whether the seemingly minimal acute damage that occurred at low doses is sufficient to induce pulmonary complications over an extended period of time (>1 month). Future studies will address this problem by monitoring perfused animals with several biochemical tests for at least 3 months after the lung perfusion.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07135-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzyme kinetics of acetylaminofluorene metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Rodney F. Minchin, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

Laboratory of Carcinogen Metabolism, Division of Cancer Cause and Prevention, National Cancer Institute

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Considerable evidence indicates that differences in metabolic processing of chemical toxins are critical in determining both species and organ sensitivity to individual compounds. The microsomal cytochrome P-450 system catalyzes the oxidation of a variety of substrates and consists of a family of cytochrome P-450 isoenzymes supported by other reductase proteins. The objectives of the present study are to characterize the cytochrome P-450 system using the carcinogen acetylaminofluorene which is oxidized in the 1, 3, 5, 7, 9 position on the fluorene ring and on the nitrogen. Further, once the pattern of acetylaminofluorene metabolism has been characterized, the effects of various inducers and inhibitors of the microsomal monooxygenase system will be investigated. Studies to date have characterized acetylaminofluorene metabolism in control rat liver microsomes and in rat microsomes following phenobarbital and TCDD induction. These studies indicated that at least 2 cytochrome P-450 isoenzymes were responsible for the formation of the 1, 3, 5, 7 and 9-OH acetylaminofluorene while N-hydroxylation appeared to only involve one distinguishable isoenzyme. The activation of acetylaminofluorene (N-hydroxylation) was induced by TCDD whereas only 7-hydroxylation was induced by phenobarbital. Further studies have investigated acetylaminofluorene metabolism in human liver microsomes and rabbit liver microsomes. Studies presently under way involve correlating the kinetics of acetylaminofluorene metabolism in rabbit liver microsomes to that of purified forms of cytochrome P-450. The characterization of lung monooxygenase is also being pursued using this substrate.

Other Professional Personnel:

Michael E. McManus and Snorri S. Thorgeirsson, Laboratory of Carcinogen Metabolism, NCI, Bethesda, Maryland

Objectives:

A considerable body of data indicate that differences in metabolic processing of chemical carcinogens are critical in determining both species and organ sensitivity to individual compounds. Therefore, characterization of pathways involved in carcinogen metabolism as well as definition of the regulatory control of these processes appear essential for a comprehensive description of the carcinogenic process. Since cytochrome P-450 dependent oxidation may be important for activation and/or detoxification of many chemical carcinogens, considerable effort has been directed towards identifying these isoenzymes of the cytochrome P-450 system that catalyze these reactions.

The cytochrome P-450 mediated metabolism of acetylaminofluorene involves oxidation at both nitrogen and carbon atoms; the former leads to metabolic activation while hydroxylation at the 1-, 3-, 5-, 7-, and 9- positions on the fluorene ring are considered detoxification pathways. Experiments with purified rabbit hepatic cytochrome P-450 and rodent liver microsomes indicate that several isoenzymes may be involved in the metabolism of AAF. The relative importance of pathways leading to either metabolic activation or detoxification of AAF will depend on the K_m values of each isoenzyme involved.

The objectives of the present study are: 1) characterize the oxidative metabolism of acetylaminofluorene in rat, rabbit and human microsomes, 2) study the effect of selective induction of the cytochrome P-450 system in rat liver, 3) compare the kinetics of acetylaminofluorene metabolism in rabbit liver to that of purified isoenzymes, 4) study the distribution of the cytochrome P-450 system in rat and rabbit lung microsomes and isolated purified cell-types.

Methods Employed:

Microsomal preparations of rat, rabbit and human liver were prepared by 120,000 g centrifugation. The metabolites of acetylaminofluorene were quantified by high pressure liquid chromatography.

Major Findings:

The metabolism of 2-acetylaminofluorene has been studied in rat liver microsomes over a concentration range of 0.02-300 μM, and kinetic parameters determined for five oxidative pathways. The N-hydroxylation of acetylaminofluorene was best described by a single enzyme system. Pretreatment of animals with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) caused a marked induction of N-hydroxylase activity while phenobarbital had no effect. Biphasic kinetics for the 7-hydroxylation of acetylaminofluorene were observed in both control, TCDD and phenobarbital induced microsomes indicating the involvement of at least 2 cytochrome P-450 isoenzymes.

Biphasic kinetics were also observed for the 5-, 3- and 1-hydroxylations of acetylaminofluorene in control and phenobarbital induced microsomes, but in TCDD pretreated microsomes only 1-hydroxylation exhibited biphasic kinetics. TCDD caused a marked induction of these metabolic pathways while phenobarbital had no effect. Non-classical kinetics were observed for the 9-hydroxylation of acetylaminofluorene and at high substrate concentrations detoxification via this pathway and 7-hydroxylation predominated. However, at low concentrations the metabolic activation of acetylaminofluorene via N-hydroxylation was a major pathway. These data indicate that multiple forms of cytochrome P-450 are involved in acetylaminofluorene metabolism and that the balance between metabolic activation and detoxification of this substrate is dependent both on concentration and previous exposure to inducers.

The kinetics of 2-acetylaminofluorene metabolism have been studied in liver microsomes from four human subjects. The N-hydroxylation of acetylaminofluorene was best described by a single enzyme system while biphasic kinetics for the 7-hydroxylation of acetylaminofluorene in all four subjects were observed and a two enzyme system best described the data. The 3- and 5-hydroxylation of acetylaminofluorene were minor metabolic pathways. Non-classical Michaelis-Menten kinetics were observed for the 9-hydroxylation of acetylaminofluorene and at high substrate concentrations this was the major metabolite formed. These data demonstrate the involvement of at least two forms of human cytochrome P-450 in the metabolism of acetylaminofluorene.

Significance to Biomedical Research and the Program of the Institute:

Better kinetic information concerning the activation of the carcinogen, AAF, will more clearly define the biochemical/molecular mechanisms involved in cancer induction by this type of agent. Increased molecular-level understanding of carcinogenesis may contribute to the rational development of intervention and/or treatment of the disease.

Proposed Course:

Studies are presently under way using rabbit microsomes and purified cytochrome P-450 isozymes to study acetylaminofluorene. Similar studies are being undertaken in rat and rabbit isolated hepatocytes. The future course of this project is to apply the technique of cytochrome P-450 characterization with acetylaminofluorene to purified lung cell populations.

Publications:

1. McManus, M.E., Minchin, R.F., Sanderson, S., Wirth, P.J. and Thorgeirsson, S.S.: Kinetic evidence for the involvement of multiple forms of human liver cytochrome P-450 in the metabolism of acetylaminofluorene. Carcinogenesis, 1983, in press.

2. McManus, M.E., Minchin, R.F., Wirth, P.J. and Thorgeirsson, S.S.: Kinetics of N- and C-hydroxylation of 2-acetylaminofluorene in rat liver microsomes. Cancer Res., 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07136-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Localized production of reduced oxygen species in the lung

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Rodney F. Minchin, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.15	0.15

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In the lung, several enzyme systems including monooxygenase activity have been found to be localized within discrete cell populations. Consequently, several pulmonary toxins are particularly damaging to only select cell-types. In order to study where reduced oxygen species such as superoxide or hydrogen peroxide are produced within the lung, a histochemical technique utilizing the heavy metal, cerium, has been investigated. Cerium reacts with superoxide/hydrogen peroxide to produce a precipitate that can be readily visualized by electron microscopy. The technique has been applied mainly in rat lung slices using nitrofurantoin or high oxygen tensions as a source of reduced oxygen. Recent studies have also used isolated perfused rat lung and isolated rat lung cells to investigate localized production of superoxide. Extensive studies using Ce/H_2O_2 in the presence of a range of antioxidant enzymes and superoxide traps were undertaken in order to characterize the interaction of cerium with reduced oxygen. The rate of this reaction was biphasic, pH-dependent and inhibited by ascorbic acid, superoxide dismutase and albumin but not by ethanol or mannitol. In rat lung slices, cerium-derived electron dense bodies were seen principally around the alveolar type II cells. These bodies were diminished in the presence of catalase but markedly increased by superoxide dismutase. Studies are presently under way to quantify the precipitated cerium under various conditions using X-ray analysis and labeled cerium.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Anthony A. del Campo, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

The production of superoxide has been suggested as the prime cause of lung toxicity induced by several compounds such as mitomycin C, paraquat and nitrofurantoin. Because the activation of several toxins including the furan and naphthalene derivatives appears to be localized in discrete lung cell populations, it was of interest to investigate whether the enzyme systems capable of generating superoxide and other toxic products of molecular oxygen are also localized within the lung.

Previous observations indicated that the heavy metal, cerium, precipitated in the presence of hydrogen peroxide and/or superoxide, forming electron-dense deposits that can be readily visualized by electron microscopy. Therefore, a study was undertaken to investigate whether this technique could be applied to our studies in the lung. The aims of the project are: 1) to characterize the chemical interaction of cerium with hydrogen peroxide/superoxide and 2) to establish the conditions under which cerium precipitation could be used to study localized production of reduced oxygen species *in vitro* and *in vivo*.

Methods Employed:

Rat lung slices were incubated in the presence or absence of various enzymes and antioxidant agents for 15 min at 37°C. $CeCl_3$ was added and the slices were further incubated for an additional 60 min. The slices were fixed and prepared for electron microscopy. Rat lungs were also perfused *in situ* for 60 min under different oxygen tensions following which the tissue was prepared for electron microscopy.

Major Findings:

The chemical interaction of cerium with hydrogen peroxide produced a characteristic product that could be monitored at 340 nm. The rate of this interaction was biphasic and pH dependent. At a pH of 7 or greater, a flocculant off-white precipitate was produced. The interaction of cerium with hydrogen peroxide was inhibited by ascorbic acid, superoxide dismutase, and albumin.

Incubation of rat lung slices with cerium resulted in electron-dense deposits principally localized on the surface of the type II cells. Preincubation with nitrofurantoin appeared to increase the occurrence of cerium-related precipitation although exact quantitation of the extent of precipitation has proved elusive. Precipitation was enhanced by the addition of superoxide dismutase

but completely inhibited by catalase suggesting that hydrogen peroxide may be the prime oxygen species involved.

Studies with perfused rat lungs using various oxygen tensions have indicated that, during a one hour experiment, very little cerium diffuses out of the capillary vasculature. Electron-dense deposits were only evident in the vasculature and only when the albumin in the perfusate was replaced with sucrose. Whole animal studies completed to date have not been successful in producing cerium deposits within the lungs.

Significance to Biomedical Research and the Program of the Institute:

The cellular localization of superoxide production in lung may have major implications for target cell-directed cytotoxicity of certain classes of chemical compounds. Further knowledge of the cellular sites of reactive oxygen production will lead to increased understanding of the pathogenesis of lung injury by certain drugs, including some that are used in cancer chemotherapy.

Proposed Course:

The future course of this project is to investigate techniques for reducing the background precipitation present in the control studies and to attempt to quantify the extent of precipitation using labeled cerium.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07137-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacokinetics of doxorubicin in isolated perfused lung of dog and man

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Rodney F. Minchin, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project investigates the feasibility of treating non-resectable pulmonary cancers by perfusing the lungs in isolation with high concentrations of anticancer agents. A study was initially undertaken to investigate the kinetics of doxorubicin, an anticancer agent active against sarcoma, in in situ perfused dog lungs. The procedure entailed inserting cannulae into the left pulmonary artery and left venous return, effectively isolating the left lobes from the systemic circulation. A wide range of perfusate doxorubicin concentrations was studied in order to determine the rate of drug uptake and retention in the lung tissue. Drug levels were determined using a specific high pressure liquid chromatographic assay. The kinetics of doxorubicin in the dog lungs were analyzed by modeling the data to a physiologically representative model of the perfusion technique. Doxorubicin was concentrated in dog lung during a 50 min perfusion. The uptake of drug increased with time but was saturable at perfusate concentrations exceeding 20 nmol/ml. Changes in the model parameters suggested that, at high perfusate concentrations, doxorubicin influx was inhibited. These studies have lead to a Phase I clinical trial utilizing the hemiperfusion technique in metastatic sarcoma patients. The surgical and perfusion procedures were successfully performed in all the patients examined to date. However, in human lung, doxorubicin accumulation was considerably slower than in the dogs. The concentration of drug in biopsied tumors from the patients was consistently less than the surrounding tissue.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel:

Michael R. Johnston, Surgery Branch, NCI, Bethesda, Maryland

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Michael A. Aiken, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Richard Terrill, Surgery Branch, NCI, Bethesda, Maryland

Objectives:

Lung perfusion was proposed as a possible procedure for the treatment of lung tumors which were surgically unresectable and unresponsive to conventional chemotherapy. Of major interest initially were patients developing pulmonary metastases from soft-tissue sarcomas or osteogenic sarcomas, as these cancers frequently metastasize exclusively to the lungs. Preliminary studies in dogs indicated that, from the surgical point of view, isolated lung perfusion was a tenable and reproducible procedure and suggested that the method could be adapted to humans. Furthermore, doxorubicin, a drug active against sarcomas, could be perfused through the lungs for up to 60 min, at levels exceeding that achieved following intravenous administration, without causing lethal lung damage. Studies were undertaken to: a) establish an *in situ* isolated perfused dog lung preparation that could be used to study the disposition and toxicity of anticancer agents in lung, b) investigate the pharmacokinetics of doxorubicin in the dog lung model and c) to establish a phase I clinical trial utilizing this procedure in cancer patients.

Methods Employed:

The pharmacokinetics of doxorubicin was investigated in the lungs of dogs and three pulmonary cancer patients perfused *in situ*. Perfusion of the left pulmonary lobes was performed using homologous blood as the perfusate and varying concentrations of doxorubicin (0.5-120 nmol/ml in dogs and 1-2 nmol/ml in humans). Cannulae were placed in the left pulmonary artery and left venous return, essentially isolating the circulation of the left lobes. Lungs were perfused for 50 min, during which time reservoir blood samples and lung tissue biopsies were collected for drug analysis using a specific high pressure liquid chromatographic procedure.

Major Findings:

In the dogs, doxorubicin uptake increased with time but was saturable at perfusate concentrations above 20 nmol/ml. After 50 min, tissue levels were consistently higher than blood perfusate levels. The kinetics of doxorubicin uptake were well described by a simple diffusion model. Changes in the estimated kinetic model parameters suggested that the saturation of doxorubicin

accumulation at higher concentrations was due to a decrease in the relative rate of uptake rather than a change in the rate of efflux. In the 3 human patients studied, doxorubicin accumulation in the perfused lung was considerably slower compared to dog lung and the level of drug measured in human lung tumor biopsies was consistently less than surrounding tissue. The present study has shown that lung perfusion, as a means to deliver cytotoxic drugs to unresectable pulmonary tumors in man, is technically feasible. In both dog and man, the level of DOX that can be attained during a 50 min perfusion is considerably higher than that expected following systemic administration of the drug.

Significance to Biomedical Research and the Program of the Institute:

This project is designed to define a new and better approach to treat certain very difficult cancers in man. In addition, the methodology and the approaches taken will yield much new basic scientific information concerning pulmonary drug uptake, metabolism, efflux and pharmacokinetics.

Proposed Course:

The future objectives of this project are: 1) to establish an *in situ* whole lung perfusion technique requiring complete cardiopulmonary bypass, 2) examine the kinetics and toxicity of doxorubicin during complete bypass, 3) manipulate selected physiological and biochemical parameters that may permit a low incidence of adverse drug effects under these conditions, and 4) extend the model to other candidate agents.

Studies addressing these problems are presently under way.

Publications:

1. Johnston, M.R., Minchin, R.F., Schull, J.H., Thenot, J.P., McManus, B.M., Terrill, R. and Boyd, M.R.: Isolated lung perfusion with doxorubicin: a preclinical study. *Cancer*, in press.

2. Minchin, R.F. and Boyd, M.R.: The uptake and metabolism of doxorubicin in isolated perfused rat lung. *Biochem. Pharmacol.*, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07138-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Distribution, metabolism and covalent binding of methyl-CCNU in rats

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Robert A. Kramer, Guest Worker

Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies completed, under way, or planned include investigations of the role of in vivo metabolism, distribution and tissue macromolecular binding of MeCCNU, in mediating the nephrotoxicity of MeCCNU in Fischer 344 rats. Ongoing studies are designed to explore the effects of drug metabolism inducers and inhibitors, as well as the effect of depletion of tissue glutathione levels on the covalent binding, disposition and toxicity of MeCCNU. Preliminary experiments have also addressed the role of the carbamoylating and alkylating activity of MeCCNU in relation to this compound's nephrotoxicity. Initial studies were conducted in Fischer rats administered MeCCNU labeled either within the carbamoylating [(cyclohexyl-1-^{14}C)-MeCCNU] or alkylating [(2-chloroethyl-1,2-^{14}C)-MeCCNU] region of the compound. These studies show fat to accumulate the highest tissue levels (nmoles/g wet weight) of parent compound (determined by HPLC analysis of tissue extracts). Kidney accumulated the highest tissue levels of the more polar ether and methanol extractable metabolites and/or degradative products of MeCCNU. Moreover, carbamoylation of tissue protein by (cyclohexyl-1-^{14}C)-MeCCNU and alkylation of DNA by (2-chloroethyl-1,2-^{14}C)-MeCCNU were higher in kidney than in any other tissue. Thus, as yet, no clear-cut case can be made for either carbamoylation or aklylation for MeCCNU-induced nephropathy. Further studies are needed to examine the metabolism, distribution and covalent binding of chloroethyl-1,2-^{14}C- and cyclohexyl-1-^{14}C MeCCNU in animals given treatments known to alter the nephrotoxicity of MeCCNU. In previous studies, we found that compounds known to induce (3-methylcholanthrene) or inhibit (piperonyl butoxide) drug metabolism provided some protective effect on the nephrotoxicity of MeCCNU. Moreover, a compound that decreased tissue glutathione levels (buthionine sulfoximine) also enhanced the nephrotoxicity of MeCCNU. Studies are now under way in which the distribution and binding of MeCCNU will be conducted in animals that have been pretreated with the aforementioned agents. The overall goals of these studies are to characterize the biochemical mechanisms underlying the nephrotoxicity of MeCCNU.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Mary G. McMenamin, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

1-(2-Chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (MeCCNU; semustine) is an investigational anticancer drug that has been widely employed in the treatment of certain advanced cancers. Preclinical toxicity studies identified the kidney as a target organ for MeCCNU toxicity. However, early clinical trials with this drug did not provide any indication of nephrotoxicity. More recently, however, a relationship between MeCCNU and a delayed irreversible and often fatal nephrotoxicity in patients has become established. A more detailed summary of the background information relevant to MeCCNU, and a description of a suitable model for studying the nephrotoxicity of MeCCNU in the Fischer 344 rat is given in a separate report (Z01 CM 07139 LETM).

In previous studies, we observed a dose-related decrease in the tubular secretion of PAH by kidney slices as early as 1 hr after MeCCNU administration. However, MeCCNU did not inhibit the uptake of PAH when incubated directly with kidney slices for 90 min. This suggested that neither the parent compound nor a spontaneous breakdown product of the nitrosourea was acting as a direct inhibitor of the anion transport system. MeCCNU is known to decompose spontaneously in aqueous solutions to form carbonium ion alkylating agents as well as isocyanates that are capable of carbamoylation reactions. MeCCNU also is known to be metabolized in the liver by a cytochrome P-450 dependent reaction and pretreatment of mice with SKF-525A, a mixed function oxidase inhibitor, reportedly increases the lethality and bone marrow toxicity of MeCCNU.

More detailed and specific analyses of the formation, distribution and covalent binding of metabolites and degratory products of MeCCNU in various tissues in a relevant in vivo model is needed. To achieve these goals, tissue distribution and binding studies were conducted in Fischer rats administered MeCCNU labeled either within the carbamoylating ([cyclohexyl-1-^{14}C]-MeCCNU) or alkylating ([2-chloroethyl-1,2-^{14}C]-MeCCNU) region of the compound.

Major Findings:

Analysis of tissue extracts by high-pressure liquid chromatography (HPLC) showed fat to accumulate the highest tissue levels (nmoles/g wet weight) of parent compound, whereas kidney accumulated the highest tissue levels of the more polar ether and methanol-extractable metabolites and/or degradative products of MeCCNU. For either label, in all tissues and at all time points (1/2, 1, 2, 4, 8, 12 and 24 hr) the majority of radioactivity was extracted from tissues with methanol. Only trace amounts of parent compound or ether extractable

metabolites and/or degradative products were present in the tissues of animals administered 2-chloroethyl-1,2-^{14}C-MeCCNU, whereas ether extractable radioactivity accounted for a significant proportion of the total tissue levels of cyclohexyl-1-^{14}C-MeCCNU.

The high levels of MeCCNU and its biotransformation products in kidney would be predicted from a compound such as MeCCNU, which is excreted primarily in the urine. Moreover, these data are in agreement with other studies in which CCNU was reported to be extensively metabolized and/or degraded within minutes after administration to rats.

The kidney also is exposed to comparatively high levels of reactive intermediates of MeCCNU. In this regard, we have found that greater levels of cyclohexyl-1-^{14}C-MeCCNU was covalently bound to kidney protein at 24 hr (123 pmol/mg prot) than either liver (104 pmol/mg prot) or lung (73 pmol/mg prot). Covalent binding by MeCCNU labeled within the cyclohexyl moiety is presumably due to carbamoylation by the isocyanate of MeCCNU. It has previously been suggested by others that carbamoylation is unnecessary for the antitumor activity of the nitrosoureas and that carbamoylation may be responsible for unwanted toxic side effects to normal host tissue.

A number of previous studies have shown that the antitumor activity of MeCCNU is due to alkylation and subsequent cross-linking of nucleophilic sites in DNA by the 2-chloroethyl carbonium ion, which along with the isocyanate comprises the major reactive intermediates of MeCCNU. Our data are in agreement with those studies. We found no radioactivity to be associated with DNA when MeCCNU was administered with label in the cyclohexyl moiety. On the other hand, significant levels of radioactivity were associated with DNA when MeCCNU was labeled within the chloroethyl moiety. Moreover, DNA-bound chloroethyl-^{14}C-MeCCNU was greatest in kidney at 4 hr (5 nmol/mg DNA), followed by liver (2.7 nmol/mg DNA) and lung (1.24 nmol/mg DNA). Interestingly, protein bound chloroethyl-^{14}C-MeCCNU was greatest in lung at 24 hr (141 pmol/mg prot), followed by kidney (78 pmol/mg prot) and liver (67 pmol/mg prot). The data show that carbamoylation of protein by cyclohexyl-^{14}C-MeCCNU and alkylation of DNA by chloroethyl-1,2-^{14}C-MeCCNU were higher in kidney than in any other tissue. Thus, as yet, no clear-cut case can be made for either carbamoylation or alkylation for the nephrotoxicity of MeCCNU. Further studies are needed in which the metabolism, distribution and covalent binding of chloroethyl-1,2-^{14}C-MeCCNU and cyclohexyl-1-^{14}C-MeCCNU are conducted in animals which have been subjected to various treatments known to alter the nephrotoxicity of MeCCNU.

In previous studies, we have shown that pretreatment with compounds known to induce (3-methylcholanthrene) or inhibit (piperonyl butoxide) drug metabolism provided some protective effect on the nephrotoxicity of MeCCNU. Moveover, pretreatment with a compound that reduced tissue glutathione levels (buthionine sulfoximine) also enhanced the nephrotoxicity of MeCCNU. It is conceivable, therefore, that the nephrotoxicity of MeCCNU might be mediated by a metabolically produced electrophilic metabolite or degradation product derived therefrom. However, it should be noted that these pretreatments may modify the toxicity of MeCCNU indirectly. For example, piperonyl butoxide is excreted to a large extent in urine and may therefore alter the renal handling and/or pharmacokinetics of MeCCNU.

In any event, studies are now under way in which the identical experimental design as that outlined above will be employed in animals that have been pretreated with the aforementioned agents (e.g., 3-methylcholanthrene, phenobarbital, piperonyl butoxide, buthionine sulfoximine).

Significance to Biomedical Research and the Program of the Institute:

The nitrosoureas are an important class of alkylating agents with demonstrated activity against a variety of tumors. A problem resulting from the longer-term survival of patients receiving such therapy has been the development of previously unseen, cumulative dose-limiting toxic effects. In this regard, nephrotoxicity limits the therapeutic potential of MeCCNU, streptozotocin and possibly other nitrosoureas in clinical use (e.g., chlorozotocin and CCNU). Therefore, it is our hope that the objectives and goals described herein will result in the development of better strategies for predicting, preventing or treating the adverse reactions of these drugs in patients. In addition, such studies may contribute to the development of new nitrosoureas which are less nephrotoxic.

Proposed Course:

Future studies involve the further elucidation of the chemico-biologic events underlying the nephrotoxicity of MeCCNU. Specifically, such studies will address: a) the role of carbamoylation and/or alkylation in MeCCNU-induced nephropathy; these studies include a detailed analysis, between organs, of DNA alkylation and either the repair thereof or formation of DNA cross-links resulting from MeCCNU administration in an appropriate animal model; b) the role of metabolism in mediating the nephrotoxicity of MeCCNU; these studies will explore the possibility that a reactive form of MeCCNU may be formed in situ in kidney or formed in liver and is blood-borne to the kidney; c) the role of GSH, and GSH metabolism in mediating the nephrotoxicity of MeCCNU; and d) development of an isolated perfused kidney system for studying MeCCNU metabolism, excretion and covalent binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07139-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nephrotoxicity of nitrosoureas

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert A. Kramer, Guest Worker
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies completed, under way or planned on the target tissue toxicity of the nitrosoureas (e.g., MeCCNU, BCNU, chlorozotocin and streptozotocin) include: a) the development of in vivo models of nephrotoxicity; b)elucidation of the biochemical mechanisms underlying the delayed and progressive nephropathy of MeCCNU; c) characterization and mechanism of the acute nephrotoxicity of streptozotocin.

The initial emphasis has been primarily on MeCCNU. We have developed a reliable model of MeCCNU renal damage in BDF mice and Fischer 344 rats and have shown that histopathological changes produced by the drug are closely paralleled by marked changes in biochemical parameters (e.g., PAH transport) measurable in vitro in kidney slices, as well as by certain in vivo renal function tests (e.g., urinary osmolality and excretion of kidney-derived urinary enzymes). Preliminary studies show the Fischer rat to provide a relevant model also for studying the nephrotoxicity of other nitrosoureas. For example, streptozotocin was found to be acutely nephrotoxic, as measured by in vivo renal function tests, whereas, low doses of chlorozotocin result in a chronic progressive nephropathy. The overall goals of these studies are to: a) elucidate the chemico-biologic events that underlie the nephrotoxicity of the nitrosoureas and b) develop improved methods for predicting, monitoring or treating such reactions in patients.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Mary G. McMenamin and Michael A. Aiken, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Background:

Only recently has it become established that MeCCNU belongs to a growing class of anticancer agents which have in common a delayed, often irreversible, organ specific toxicity that is dependent upon the total cumulative dose administered. In this regard, the nephrotoxicity of MeCCNU can be compared to the pulmonary toxicity caused by the structurally related compound, bis-chloroethyl nitrosourea, or to the cardiotoxicity resulting from adriamycin therapy. Suitable animal models for the study of such cumulative dose-dependent and organ specific toxicities generally are either unavailable or have not been characterized in substantial detail, and are limited by a lack of sensitive methods for the detection of injury early in the course of the pathogenesis.

Other nitrosoureas in clinical use are streptozotocin, chlorozotocin, BCNU and CCNU. Renal toxicity was noted in preclinical studies for all of the above compounds, with streptozotocin being the most nephrotoxic and BCNU the least nephrotoxic. A specific mechanism of nephrotoxicity has not been implicated for any of these nitrosoureas, nor is it known if the different derivatives are likely to cause kidney injury by the same process. However, further insight into the mechanism(s) might help answer several intriguing questions concerning the current group of clinically important nitrosoureas. For example, why does MeCCNU damage primarily the kidneys and not the lungs, whereas the reverse is true for BCNU? Also, among the nephrotoxic nitrosoureas, why do some (e.g., streptozocin) appear to cause alterations in renal functions very acutely, whereas others (e.g., MeCCNU, CCNU) seem to cause delayed kidney damage? The available literature contains few hints to answer these questions. Moreover, the above differences derive principally from clinical observations, and the demonstration and experimental analysis of their counterparts in appropriate animal models has not previously been accomplished.

Objectives:

Initial experiments in developing an appropriate animal model for the study of MeCCNU-induced nephrotoxicity were conducted in BDF mice and were described in detail in the FY 1982 Annual Report (Z01 CM 07125-01 LETM). In these studies, MeCCNU was found to be nephrotoxic and to produce dose-related histopathological changes that closely paralleled changes in biochemical parameters (e.g., PAH and TEA transport) measurable *in vitro* in biopsy specimens. In order to extend our model to one that would permit the evaluation of renal function *in vivo*, we have sought to develop a model of MeCCNU nephrotoxicity in male Fischer 344 (F344) rats utilizing sensitive *in vitro* and *in vivo* methods for monitoring renal function.

Major Findings:

We found MeCCNU to be nephrotoxic to F344 rats at doses, 20-80 mg/kg (120-480 mg/M^2), that approximate the typical human therapeutic dose (200 mg/M^2). The most sensitive indicator of MeCCNU induced nephrotoxicity was found to be the impaired accumulation of the organic anion PAH, but not of the organic cation TEA, by kidney slices in vitro. The decrease in PAH accumulation occurred within 1-12 hr after treatment and with doses as low as 20 mg/kg (120 mg/M^2) MeCCNU. PAH uptake was decreased for as long as 28 days after a single administration of either 40 mg/kg or 80 mg/kg MeCCNU. Furthermore, the decrease in PAH uptake was dose-related at both 24 hr and 28 days.

The relatively selective effect of MeCCNU on organic anion accumulation suggests the possibility that the drug causes pathological alterations at discrete regions along the nephron. For example, the straight descending portion of the proximal tubule (pars recta), has been estimated to account for approximately 75% of the total anion secretory capabilities of the rabbit nephron, whereas, cations are believed to be transported by a separate mechanism in many species.

The presence of abnormally high levels of renal enzymes in the urine is evidence of a break in renal cellular integrity and has been established as an approach to detect a number of drug-induced nephrotoxicities. MeCCNU increased the urinary excretion of lactate dehydrogenase (LDH) and N-acetylglucosaminidase (NAG), however, not before days 3-6 after dosing. The delay between the first evidence of renal damage (decreased PAH uptake) and appearance of enzymuria may correlate with the time required for the initial insult to result in cellular necrosis and/or gross cellular abnormalities. In support of such a relationship was the inverse correlation between the dose-dependent decrease in PAH accumulation observed in kidney slices at 24 hr (r^2 = 0.92) and increased urinary excretion of LDH and NAG observed on day 6 (r^2 = 0.88, for either NAG or LDH). Furthermore, abnormal changes in other measures of renal function were also delayed (e.g., polyuria, loss of urine concentrating ability and proteinuria) and in some cases (increased LDH excretion, alkalinuria, and decreased urine concentrating ability) progressed in severity throughout the duration of the experiment. These data suggest that the changes in renal function observed 3-28 days after MeCCNU administration may occur secondarily to an initial lesion in the straight descending portion of the proximal tubule (pars recta). The biochemical alterations described in the present investigation are in agreement with histopathologic observations, at the light microscopic level, of renal damage that first became evident several days after dosing and then progressed to involve greater regions of the nephron.

The progressive changes in renal histology, chronic impairment of anion transport, and increased urine pH and LDH excretion occurred without an increase in BUN levels. This observation only serves to illustrate the insensitivity of the latter measure of renal integrity, and may provide an explanation for the delayed appearance of elevated BUN or serum creatinine levels in patients receiving MeCCNU. The potentially irreversible nephrotoxic effect of a single dose of MeCCNU indicates that the F344 male rat appears to be a good model for studying the mechanism of MeCCNU, and perhaps the effect of other nitrosoureas that may be nephrotoxic to humans.

Preliminary studies indicate that the model described herein, for MeCCNU, may be appropriate for studying the nephrotoxicity of other nitrosoureas. For example, renal slice uptake studies in animals pretreated with the aforementioned nitrosoureas showed MeCCNU to be the most effective at decreasing PAH uptake, whereas BCNU has the least effect. In contrast, streptozotocin treatment actually increased PAH uptake. The enhanced uptake of PAH caused by streptozotocin is not without precedent. Other nephrotoxins such as gentamicin produce a similar effect on kidney slice transport processes. In addition, only streptozotocin was acutely nephrotoxic, as judged by renal function tests. These results correlate well with clinical observations.

Of considerable interest has been the finding that chlorozotocin causes a chronic progressive nephropathy, similar to that found following MeCCNU administration. Chlorozotocin differs structurally from MeCCNU by substitution of a glucose molecule for the cyclohexyl ring of MeCCNU resulting in the lowest carbamoylation activity of any of the chloroethyl nitrosoureas. The similarity in the nephrotoxicity produced by these two nitrosoureas may suggest that alkylation by the chloroethyl moiety, and not carbamoylation by the isocyanate of MeCCNU, may be responsible for the chronic progressive nephropathy associated with both compounds. A more detailed evaluation of the role of carbamoylation vs. alkylation in the target organ toxicity of MeCCNU will be discussed in an accompanying report (Z01 CM 07138-01 LETM).

Significance to Biomedical Research and the Program of the Institute:

Only through an increased understanding of the biochemical mechanisms involved in drug-induced nephrotoxicity can the prevention and/or treatment of such reactions be more rationally approached. Nephrotoxicity is a major limitation on the therapeutic indices of a number of otherwise very useful anticancer drugs. Therefore, better approaches to limiting the nephrotoxicity may result in substantially improved clinical value of such drugs.

Proposed Course:

Attention will be directed at the further elucidation of the mechanism(s) of MeCCNU nephrotoxicity in this acute/subacute model as well as toward the development of a chronic, repetitive-dosing model that may more closely mimic the clinical situation.

Future experiments also will be designed to explore strategies for minimizing or preventing MeCCNU-induced nephropathy by the following manipulations: hydration diuresis; thiol protective agents; radioprotectors; and inhibition of renal organic ion transport. In addition, we plan to investigate the possible additive and/or synergistic nephropathic effects produced by multiple dosing of MeCCNU, and of other anticancer drugs (e.g., adriamycin) used in conjunction with MeCCNU or other presumably less nephrotoxic nitrosoureas. We shall also attempt to develop a chronic model of MeCCNU nephropathy using multiple-dose regimens and/or combinations with other drugs or conditions that may predispose to development of chronic nephropathy with histologic charac-

of renal fibrogenesis will be investigated. Studies addressing other chemicobiologic conditions necessary for the susceptibility of kidney towards nitrosourea-induced nephropathy (i.e., renal metabolism, covalent binding, GSH conjugation, age and sex differences) will be discussed in an accompanying report (Z01 CM 07138-01 LETM).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07140-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of BCNU on pulmonary glutathione reductase

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Adaline C. Smith, Guest Worker

Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

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SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

BCNU, a widely-used antitumor drug, has previously been reported to cause irreversible inhibition of glutathione (GSSG) reductase in liver and red blood cells. GSSG reductase is responsible for maintaining the intracellular glutathione redox status and thereby has a major role in protecting the cell from oxidant damage. Because one of the most prominent clinical side-effects of BCNU is pulmonary toxicity, we have examined the effects of the drug on pulmonary GSSG reductase and GSH/GSSG ratio. A. In Vivo Studies: Single doses of 8 mg BCNU/kg or 80 mg BCNU/kg inhibited lung GSSG reductase by 11% and 82%, respectively. The inhibition was very persistent, lasting up to 7 days after a single dose of BCNU. The effect of multiple doses of BCNU on GSSG reductase and GSH/GSSG levels was also studied. The multi-dosing regimen of 5 mg BCNU/kg/week for 6 weeks results in the development of severe pulmonary damage. After 3 weeks of BCNU dosing, GSSG reductase activity decreased to 50% of control values and GSSG levels rose to 227% of control values. After 6 weeks of BCNU, GSSG reductase activity decreased to 22% of control values. B. In Vitro Studies: BCNU also inhibited pulmonary GSSG reductase in vitro. The inhibition was dependent on length of incubation time and the concentration of BCNU. Other nitrosoureas, methyl-CCNU and CCNU, also were able to inhibit GSSG reductase while streptozotocin and chlorozotocin had little if any effect on GSSG reductase activity. The in vitro inhibition can be mimicked by N-ethylmaleimide which binds to free sulfhydryl groups. Addition of sulfhydryl donors, such as GSH and dithiothreitol, into the incubation media prevents the inhibition of GSSG reductase by BCNU. The glutathione redox system has been proposed to be important as an antioxidant defense mechanism in pulmonary cells and impairment of this system by BCNU might ultimately lead to or contribute to pulmonary toxicity.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

George Kim, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

BCNU, a potent alkylating and carbamylating agent, has been shown by others to be a potent inhibitor of glutathione (GSSG) reductase in the liver and also in red blood cells. GSSG reductase is responsible for catalyzing the conversion of glutathione disulfide (GSSG) to two molecules of reduced glutathione (GSH). Through this catalytic reduction of GSSG, GSSG reductase is responsible for maintaining the intracellular glutathione redox status and thereby has a major role in protecting the cell from oxidant damage. Studies were initiated to investigate the effect of BCNU administration on pulmonary GSSG reductase and to demonstrate whether impairment of this system by BCNU might ultimately lead to or contribute to its pulmonary toxicity.

Methods Employed:

Animals: Male Fischer 344 rats weighing about 150 grams were allowed food and water ad libitum. All the nitrosoureas used in these studies were supplied by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. BCNU was dissolved in sesame oil and injected intraperitoneally. Rats were sacrificed by a pentobarbital overdose (60 mg/kg). Lungs were perfused in situ with heparinized saline until the tissue was blood-free. The lungs were then excised, rinsed, trimmed and weighed.

Assays: Lung tissue was homogenized in 4 volumes of 0.25 M sucrose in 50 mM Tris (pH 7.4). For GSSG reductase activity, an aliquot of homogenate was centrifuged at 105,000 xg. The cytosol fraction was assayed for enzymatic activity. The incubation buffer consisted of 1 mg cytosolic protein, 0.3 µmol GSSG, 0.1 µmol NADPH, 3 µmol EDTA, 2 mg BSA in 1 ml 50 mM potassium phosphate buffer (pH 7.6). Disappearance of NADPH was monitored at 340 nm. Purified yeast GSSG reductase was used to establish a standard curve. Glutathione (reduced and oxidized) was determined by the glutathione reductase assay method. The protein in one ml of lung homogenate was precipitated with 5% trichloroacetic acid. The protein was pelleted by centrifugation and supernatant was assayed for GSH. GSSG was assayed with the addition of 0.1 M N-ethylmaleimide (NEM). The excess NEM was exhaustively extracted with diethyl ether. The GSH and GSSG was then assayed by a published procedure.

Major Findings:

A. In Vivo Studies

Single doses of BCNU markedly reduce lung GSSG reductase activity. A single

dose of 8 mg BCNU/kg inhibits GSSG reductase by 11% and 80 mg BCNU/kg inhibits the enzyme by 82%. This inhibition occurs within one hour after dosing and is quite persistent, lasting up to 7 days after the administration of BCNU. A preliminary study indicated that the multi-dosing regimen with BCNU which causes pulmonary damage also results in marked alterations of glutathione and glutathione reductase. A single dose of 5 mg BCNU/kg resulted in only minimal inhibition of pulmonary GSSG reductase activity. However, after 3 doses of 5 mg BCNU/kg/week (total cumulative dose of 15 mg/kg) resulted in a 50% inhibition of GSSG reductase and a substantial (227%) increase in intracellular GSSG levels. After 6 doses of BCNU (total cumulative dose of 30 mg/kg), the GSSG reductase levels were inhibited by 78%.

B. In Vitro Studies

In vitro BCNU also potently inhibits GSSG reductase in 105,000 x g pulmonary cytosol. The inhibition is dependent upon incubation time and concentration of BCNU. Other nitrosoureas, methyl-CCNU and CCNU, were also able to inhibit pulmonary GSSG reductase in vitro but these nitrosoureas were not as potent as BCNU. Streptozotocin and chlorozotocin had little if any effect on lung GSSG reductase activity. The in vitro inhibition of pulmonary GSSG reductase can be mimicked by compounds which bind to free sulfhydryl groups such as N-ethylmaleimide. Addition of sulfhydryl donors, such as GSH and dithiothreitol, into the incubation media prevent the inhibition of GSSG reductase by BCNU. These data suggest that perhaps BCNU (or one of its metabolites) is binding to an essential sulfhydryl group on pulmonary GSSG reductase. Interestingly, GSSG, the substrate for GSSG reductase, prevents the inactivation of the enzyme by BCNU. This protection does not appear to be dependent on the generation of GSH since GSSG in very small concentrations completely prevented BCNU-induced inhibition, whereas the appropriate concentrations of GSH only delayed the inhibition of the enzyme by BCNU. These data suggest perhaps BCNU is inactivating GSSG reductase at or near the GSSG binding site.

Significance to Biochemical Research and the Program of the Institute:

We are attempting to define the pulmonary toxicity of BCNU in an appropriate animal model and possibly identify the mechanism by which BCNU induces pulmonary damage. We have demonstrated that BCNU markedly affects pulmonary GSSG reductase both in vivo and in vitro. Since GSSG reductase is an important enzyme in the maintenance of GSH/GSSG levels, impairment of this vital antioxidant defense mechanism by BCNU may lead to or contribute to its pulmonary toxicity.

Proposed Course:

Current studies are aimed at the further exploration of the possible relationship of GSSG reductase inhibition by BCNU to the development of pulmonary toxicity in the multi-dose model. A study is currently under way to investigate in greater detail, the temporal changes of GSSG reductase as well as changes in the GSH/GSSG ratio during and after BCNU dosing and how these changes relate to pulmonary damage, as measured by pulmonary and serum ACE and lung histopathology. These studies should give a clearer understanding of the relationship of GSSG reductase inhibition by BCNU and the development of pulmonary toxicity.

Further in vitro studies include attempts to partially purify pulmonary GSSG reductase to closely study the kinetics of inhibition by BCNU in more detail and how to perturb this inhibition.

Publications

1. Smith, A.C. and Boyd, M.R.: Mechanisms of drug-induced pulmonary toxicity. Trends Pharmacol. Sci. 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07141-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of BCNU on pulmonary and serum angiotensin converting enzyme

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Adaline C. Smith, Guest Worker

Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.5	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The anticancer drug, BCNU, causes potentially life-threatening lung injury in a high percentage of patients. Because changes in the activities of pulmonary and serum angiotensin converting enzyme (ACE) have been previously reported to reflect toxic pulmonary damage by certain agents, we have investigated the effects of BCNU on pulmonary and serum ACE in F344 rats. In vitro, BCNU had a direct inhibitory effect on both serum and pulmonary ACE. This inhibition in vitro was dependent both on time and the concentration of BCNU. In vivo, a large single dose (80 mg/kg, i.p.) did not alter pulmonary ACE nor cause histologically observable acute lung damage. However, serum ACE dropped by 25% within 1 hour after BCNU. A multidose regimen, consisting of 5 mg BCNU/kg once per week for 6 weeks, caused marked pulmonary injury, which continued to develop in severity over several weeks following the completion of dosing. Lung ACE after 4 doses (total of 20 mg BCNU/kg) was depressed by 40% and remained low until dosing was completed. Following the final dose, lung ACE returned to control within 2 weeks. However, after an additional 2 weeks, both lung and serum ACE had decreased by 35% and 25%, respectively and remained low for the duration of the study. It appears that ACE may provide a useful biochemical monitor for BCNU-induced pulmonary toxicity, but careful attention must be given to the time course of changes in the enzyme activity. ACE as a marker for pulmonary injury can be used not only to monitor the development of lung damage but also can be used to screen potential protectors against the lung damage caused by BCNU administration.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

George Kim, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

The antitumor activity of bis-chloronitrosourea (BCNU) has been found useful in combination with other chemotherapeutic agents in the treatment of a variety of malignancies and as the sole therapeutic agent in the treatment of brain tumors. In the past few years, there have been numerous reports linking BCNU administration with progressive pulmonary disease. The pulmonary toxicity of BCNU in patients is dependent upon the total cumulative dose of BCNU administered and it has been estimated 10-30% of patients treated with high doses of BCNU develop pulmonary toxicity.

Recently our laboratory has established a BCNU dosing regimen in laboratory animals which results in pulmonary damage. A dose of 5 mg BCNU/kg/week for 6 weeks to F344 rats led to diffuse pulmonary damage which is evident 14 weeks after the first dose of BCNU. Other investigators have demonstrated that serum and pulmonary ACE is useful in monitoring the pulmonary toxicity of certain xenobiotics. ACE is believed to be located on the outer membrane of capillary endothelial cells and most ACE activity is found in pulmonary tissue. Because changes in the activities of pulmonary and serum ACE seem to reflect toxic pulmonary damage, we have investigated the effect of BCNU on pulmonary and serum ACE in F344 rats.

Methods Employed:

Animals: Male F344 rats (120-170 g) were used throughout these studies. BCNU was supplied by Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. For multiple dosing experiments, the appropriate amount of BCNU was dissolved in 1 volume of ethanol and then diluted with 9 volumes of distilled water to give a final BCNU concentration of 0.5 mg/ml. For single-dose studies, in which larger doses of BCNU were used, it was necessary to administer BCNU in sesame oil vehicle because of the drug's limited solubility in other media. Animals were sacrificed by a pentobarbital overdose and blood samples were taken by cardiac puncture. Lungs were perfused *in situ* with heparinized saline, removed, trimmed, weighed and frozen at -70°C until assayed.

Angiotensin converting enzyme assay: Serum ACE was measured by the method of Rohrbach (*Anal. Biochem.* 84: 272, 1978) using (^{14}C-glycine) hippuryl-histidyl-leucine (New England Nuclear, Boston, MA). Pulmonary ACE was determined in lung homogenates as described by Newman et al. (*Cancer Res.* 40: 3621, 1980). The solubilization of the membrane-bound pulmonary ACE was accomplished by

adding 0.05% Nonidet P-40 (Sigma Co., St. Louis, MO), a procedure that released 95% of the total particulate activity. The specificity of the substrate for ACE was determined by using the specific inhibitor, Captopril, in the assay. Virtually 99% of ACE activity was inhibited by 0.2 µM Captopril in both the lung and serum enzyme assays.

The in vitro effect of BCNU on ACE activity was determined by preincubating serum or solubilized lung homogenate with the drug at 37°C. After the preincubation period, ACE activity was determined using the standard assay.

Major Findings:

Initial in vitro studies demonstrated that BCNU had a direct inhibitory effect on both pulmonary and serum ACE. Pulmonary ACE activity dropped 60% and serum ACE dropped 57% after one hour incubation with 4 mM BCNU. The degree of inhibition was shown to be dependent both upon incubation time and the concentration of BCNU. This direct inhibitory effect of BCNU on ACE was also demonstrated after large in vivo doses of BCNU (up to 80 mg/kg). However, acutely administered doses of BCNU did not alter pulmonary ACE nor cause histologically observable acute pulmonary damage.

In contrast to a single dose of BCNU, multiple doses of this drug caused marked pulmonary damage. Pulmonary and serum ACE were monitored during the BCNU dosing period and 12 weeks after the completion of dosing. Pulmonary ACE activity dropped to 60% of control values after a total cumulative dose of 20 mg BCNU/kg. This activity remained low until the administration of BCNU was completed, after which pulmonary ACE returned to control values within 2 weeks. A secondary drop in pulmonary ACE became apparent 6 weeks after BCNU dosing was completed; the pulmonary ACE activities fell to 65% of control values and remained low throughout the remainder of the study. Serum ACE did not change during the BCNU dosing period. However, within 4 weeks after the completion of BCNU dosing, serum ACE levels dropped to 70% of control values and remained low throughout the remainder of the study.

Significance to Biomedical Research and the Program of the Institute:

Administration of multiple doses of BCNU to rats causes marked decreases of pulmonary and serum ACE. Pulmonary ACE activities are depressed during the BCNU treatment period, possibly reflecting an initial phase of pulmonary damage. After BCNU treatment was completed lung ACE levels recovered, but subsequently both the pulmonary and serum ACE became depressed. At the time the late-occurring deficiencies were observed, the lungs showed severe histopathological changes. Thus, it appears that the effect of multiple doses of BCNU on pulmonary ACE is biphasic, the initial decrease possibly representing an early phase of reversible pulmonary injury and the delayed decrease possibly corresponding to a more chronic, irreversible form of lung damage.

Proposed Course:

Further studies are aimed at better defining this model of BCNU-induced pulmonary toxicity. This study is essentially completed. ACE, as a marker for

pulmonary injury, may be useful in screening potential protectors against the lung injury caused by BCNU administration.

Publications:

1. Smith, A. C., and Boyd, M.R.: The effect of bis-chloronitrosourea (BCNU) on pulmonary and serum angiotensin converting enzyme activity in rats. Biochem. Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07142-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Isolation and characterization of reactive metabolites of methylfurans

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) V. Ravindranath, Visiting Fellow
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

Toxicology Research and Testing Program, NIEHS, NIH,
Research Triangle Park, NC

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The molecular mechanisms involved in the metabolic activation of certain cytotoxic furans, namely 4-ipomeanol, a natural product isolated from mouldy sweet potatoes, and 3-methylfuran (3-MF), an atmospheric pollutant, are being investigated. Not only does the environmental occurrence of certain furans possibly have major toxicological significance for mankind, there is also a growing interest in some furan deivatives as potential antitumor agents. Oxygen and NADPH dependent metabolic activation of these furans results in the formation of highly electrophilic metabolites which alkylate microsomal proteins.

Acetylacrolein (AA), a product of peracid oxidation of 2-methylfuran (2-MF), a naturally occuring cytotoxic furan, was investigated as a possible microsomal metabolite. Glutathione, N-acetylcysteine and cysteine adducts of AA although apparently rapidly formed, were not stable enough to allow isolation. When rat liver microsomal incubations were performed with semicarbazide (SC), NADPH and 2-MF, a metabolite was isolated by gel permeation chromatography and HPLC and was found to be identical by mass spectroscopy to the disemicarbazone of AA. Methylbutenedial, the analogous oxidative ring opened product from 3-MF was isolated as the disemicarbazone from rat liver microsomal incubation with 3-MF. Addition of SC to rat lung microsomes containing ^{3}H-3-MF, and NADPH significantly inhibited covalent binding of ^{3}H-3-MF metabolites to microsomal proteins, although a considerably greater amount of 3-MF was metabolized in the presence of SC. Coordinate with the decreased covalent binding there was the appearance of the radiolabeled semicarbazide conjugate of methylbutendial. Thus, the dialdehyde appears to be the reactive metabolite of 3-MF that is capable of binding covalently to tissue macromolecules. Moreover, since the covalent binding of reactive material is directly correlated with the occurrence of toxic lesions, the dialdehyde is probably the ultimate toxic metabolite involved. Future studies will continue to explore this hypothesis and will be extended to other cytotoxic furans including 4-ipomeanol and perilla ketone.

Other Professional Personnel:

Leo T. Burka, Toxicology Research and Testing Program, NIEHS, NIH, Research Triangle Park, North Carolina

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Background:

Furans are found ubiquitously in the environment both as natural and man-made products. Furan metabolism has been of long standing interest to us in view of the pulmonary toxicity exhibited by certain furan derivatives, namely 4-ipomeanol and 3-methylfuran. 4-Ipomeanol is a natural product isolated from mouldy sweet potatoes, while 3-methylfuran (3-MF) is apparently derived from the photodecomposition of terpenes in the atmosphere. Another alkyl furan, 2-methylfuran, a constituent of cigarette smoke, causes pulmonary bronchiolar lesions in rats. Not only does the presence of certain furans possibly have major toxicological significance for mankind, there is also a growing interest in some furan derivatives as potential antitumor agents. 4-Ipomeanol has been found to be highly cytotoxic to several different human lung tumor cell lines *in vitro* and their possible *in vivo* activity is currently being explored.

There has been considerable conjecture as to how these furans exert their cytotoxic effect. Apparently, the compounds are converted to alkylating agents *in situ* within the target tissue by a cytochrome P-450 catalyzed process requiring NADPH and oxygen. The structure of the alkylating agent(s) derived from either of these furans is unknown, in fact no metabolite of any simple furan has been isolated that gives a clue as to the chemical nature of the activation process.

Objectives:

The purpose of this project is to investigate the nature of the metabolic activation of cytotoxic furans and to identify and characterize the ultimate toxic metabolites. To this end, we sought initially to study the microsomal metabolites of 2-MF and 3-MF.

Methods Employed

Metabolic activation of 2-MF which is dependent on NADPH results in the formation of highly electrophilic metabolite(s) which alkylate microsomal protein. Microsomal activation of 2-MF conceivably could lead to the formation of acetylacrolein (AA).

To test this hypothesis, chemical syntheses of AA, and relevant derivatives thereof, were undertaken. Oxidation of 2-MF with m-chloroperbenzoic acid resulted in the formation of AA.

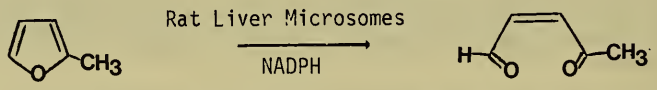
Attempts were made to synthesize glutathione (GSH) and NAC (N-acetylcysteine) conjugates of AA, as addition of either of these nucleophiles results in the reduction of covalent binding of 2-MF metabolites to microsomal proteins. Although loss of UV chromophore of AA was observed with the addition of NAC or GSH, the conjugates, due to their instability, could not be isolated. Reduction of the conjugates to the diol increased their stability and they could be analyzed on HPLC, although isolation of the corresponding metabolites from microsomal incubation was not possible.

As the glutathione and NAC adducts of reactive metabolite(s) of 2-MF were unstable, a trapping agent had to be chosen which produced a stable derivative with the reactive metabolite(s). If the reactive metabolite(s) of 2-MF were an aldehyde or ketone, it could be trapped as a semicarbazone by adding excess semicarbazide to microsomal incubations.

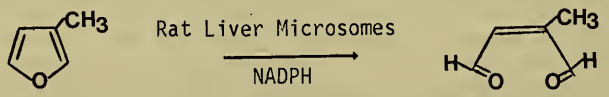
The semicarbazone of the reactive metabolite of 2-MF was isolated from microsomal incubations containing excess semicarbazide, NADPH and 2-MF by a combination of gel permeation chromatography and HPLC, and was purified by recrystallization from water. The MS of this compound was found to be identical to that of the synthetic disemicarbazone of AA.

Major Findings:

The biotransformation of 2-MF to AA by rat liver microsomes was dependent on NADPH and was inducible by pretreatment with phenobarbital.



The metabolite of 3-MF was similarly isolated from rat liver microsomal incubation containing NADPH and excess semicarbazide and was shown to be identical to the disemicarbazone of methylbutenedial.



The effect of semicarbazide on the covalent binding of ^{3}H-3-MF metabolites to rat lung microsomal proteins was also studied. Addition of semicarbazide to rat lung microsomes containing 3-MF and NADPH reduced the covalent binding to control levels (those not containing any NADPH), although high levels of alkylation of proteins was seen in incubations not containing semicarbazide. The amount of 3-MF metabolized by microsomal incubation was markedly higher in those incubations containing semicarbazide (SC) in comparison with the incubations not containing any SC. Thus, the addition of SC significantly inhibited the covalent binding of 3-MF metabolite(s) to microsomal proteins, presumably by forming a less reactive conjugate with the reactive intermediate dialdehyde.

Significance to Biomedical Research and the Program of the Institute:

Identification of acetylacrolein and methylbutenedial as the reactive metabolites of 2-MF and 3-MF provides the first insight into the nature of the activation process of toxic furans, which is of interest both in view of their ubiquitous presence in the environment and their potential antitumor activity. The novel method of trapping reactive carbonyl intermediates with semicarbazide could have wide application, as unsaturated aldehydes have already been detected or suspected as reactive intermediates for several important classes of compounds. For example, acrolein has been identified as the reactive metabolite of cyclophosphamide, both in vivo and in vitro. Similarly, there is increasing interest in the possible intermediacy of reactive aldehydic metabolites of certain carcinogenic and/or cytotoxic nitrosamines that require activation by metabolism.

Proposed Course:

Following these in vitro metabolism studies, the metabolism and covalent binding of 3-MF and 2-MF will be examined in vivo in rats. Effect of SC dosing on the metabolism and toxicity of these alkyl furans will also be studied. Isolation and characterization of the urinary metabolite(s) of these alkyl furans will be undertaken.

In addition to the studies on the methylfurans, biliary excretion of conjugates of 4-ipomeanol is being examined, with a view to isolate and characterize metabolites of 4-ipomeanol that might clarify its mechanism of activation. The bile ducts of rats were cannulated and ^{3}H-4ipomeanol (20 mg/kg) injected i.p. Bile was collected every 30 min for a period of 6 hr. In a 6 hr period, after injection of 4-ipomeanol, 15% of the total administered dose was excreted in the bile, more than 80% of it in the first half hour. Analysis of these metabolites is now in progress.

The in vitro microsomal metabolism of another toxic furan, perilla ketone, is also being studied. The synthesis of some of the possible metabolite(s) of perilla ketone has been carried out. Isolation and characterization of the in vitro microsomal metabolites of perilla ketone should give a clearer view as to the mode of activation of toxic ketofurans like perilla ketone and 4-ipomeanol.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07143-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Improved assay methodology for hydroxyfatty acids from lipid peroxidation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Leo T. Burka, Cancer Expert
Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Lipid peroxidation is a proposed mechanism for the injurious effects of reactive oxygen intermediates and many other toxins. Several methods for measuring lipid peroxidation have been described including measurement of lipofusin, conjugated dienes, aldehydes, pentane, and ethane. These methods are indirect and/or not suitable for in vivo studies. We therefore initiated an attempt to develop more direct analytical procedures for hydroxyfatty acids, which are products of lipid peroxidation, suitable for use in in vivo studies of lipid peroxidation and toxicity.

Hydroxyfatty acids were released from phospholipids by enzymatic hydrolysis. The acids were then separated by an HPLC procedure using a reverse-phase chromatography system and were detected and quantitated, against synthesized standards, by UV absorption at 235 nm.

This procedure was used successfully to demonstrate the presence of 15-hydroxyeicosatetraenoic acid and 9- and 13-hydroxyoctadecadienoic acids in livers from mice treated in vivo with necrogenic doses of carbon tetrachloride. Livers and lungs from rats given carbon tetrachloride, or from mice given paraquat, showed HPLC peaks indicative of hydroxyfatty acids, although none corresponded exactly with the synthetic standards available. Further studies will continue to probe the development and potential applicability of this methodology to the investigation of the role of lipid peroxidation in tissue injury.

Other Professional Personnel:

Walter C. Hubbard, Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tennessee

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

Lipid peroxidation is a proposed mechanism for the injurious effects of reactive oxygen intermediates and many other toxins. Several methods for measuring lipid peroxidation have been described including measurement of lipofusin, conjugated dienes, aldehydes (via the thiobarbituric acid test), pentane, and ethane. These methods are indirect and/or not suitable for in vivo studies. We therefore initiated an attempt to develop more direct analytical procedures for hydroxyfatty acids, which are products of lipid peroxidation, suitable for use in in vivo studies of lipid peroxidation and toxicity.

Methods Employed:

15-hydroxyeicosatetraenoic acid and 9- and 13- hydroxyoctadecadienoic acid standards were prepared from lipoxidase oxidation of arachidonic and linoleic acids, respectively (Gardner et al., Lipids 7: 324-334, 1972).

A procedure for isolating hydroxyfatty acids from peroxidized phospholipids has been described by Porter et al. (Lipids 15: 163-167, 1980); we used this approach as the baseline for development of our analysis. The hydroxyfatty acids resulting from enzymatic hydrolysis of phospholipids have been separated by reverse phase HPLC and detected and quantitated by UV absorption at 235 nm.

Tissue was homogenized in 20 volumes of $CHCl_3/CH_3OH$ (2:1). The homogenate was filtered and the filtrate was washed with about 1/3 volume of 0.9% saline. The aqueous layer was washed with an equal volume of $CHCl_3$; the organic layers were combined and filtered through Whatman 1PS paper. The filtrate was concentrated under vacuum and transferred to a 5 ml vial where it was dissolved in 2 ml of freshly distilled ether to which was added 50 µl of methanol, 150 µl of 0.1 M borate buffer (pH 8.5), 100 µl of 0.01 M calcium chloride, and 1 mg of phospholipase A_2 from bee venom (Sigma) in 100 µl of borate buffer. This mixture was shaken at room temperature for 1.5 hr. After this time, 100 µl of 1 M phosphoric acid was added and the mixture was extracted 3 times with 2 ml of freshly distilled ether. The ether was removed in a stream of nitrogen; the residue was taken up in 4 one ml portions of distilled hexane and loaded onto a Waters silica Sep-Pak. Then 3 ml portions of hexane/isopropanol/acetic acid (490:10:1) were used to elute the hydroxyfatty acids. The hydroxyfatty acids usually eluted in the third portion of the hexane/isopropanol/acetic acid solution. The solvent was removed under a stream of nitrogen and the residue was dissolved in 200 µl of methylene chloride followed by 1800 µl of methanol. A 50 µl portion of this solution was analyzed by HPLC using a 25 cm Rainin 5 µm C-18 column with 75% methanol, 25% water and 0.1% acetic acid at 2 ml/min as solvent. A Kratos model 773 detector was used at 235 nm.

The hydroxyfatty acids eluted in 15 to 20 min under these conditions. The minimum detectable amount of hydroxyfatty acid was ca. 1 ng as a pure standard or ca. 5 ng in a complex mixture.

Major Findings:

To determine if the procedure was suitable for quantitation of in vivo lipid peroxidation, a mouse was dosed i.p. with carbon tetrachloride. After 30 minutes, the liver was removed and processed as described above. Peaks in the HPLC trace corresponding to all three standards were found. It was determined that the liver contained 960 ng of 13-hydroxyoctadecadienoic acid, 680 ng of 15-hydroxyeicosatetraenoic acid and 1320 ng of 9-hydroxyoctadecadienoic acid.

Rats were treated with carbon tetrachloride and the lungs and livers were analyzed for hydroxyfatty acids. In these samples, although there were peaks in the 15 to 20 minute retention range, none corresponded exactly to the standards upon coinjection.

Lungs and livers from mice treated with paraquat were processed as described. Again, there were peaks in the 15 to 20 min range but none corresponded exactly to the three standards.

Significance to Biomedical Research and the Program of the Institute:

The role of lipid peroxidation in chemical and drug-induced toxicity has been an extremely controversial issue for over the past twenty years. A major part of the difficulty has arisen from the inadequacy of analytical methodologies available, and therefore any improvement in assay methods applicable especially to in vivo investigations could be pivotal to clarifying this important issue. With particular regard to anticancer drugs, there are several major classes for which membrane peroxidation is suspected or proposed to underlie toxicity and/or antitumor activity, but such correlations have been exceedingly difficult to show definitively, again due to inadequacies of the older methodologies used.

Proposed Course:

Further studies will continue to probe the continued development and the potential applicability of this methodology to the investigation of the role of lipid peroxidation in tissue injury.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07144-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunological characteristics of beige-nude mice; utility for cancer research

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

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Veterinary Resources Branch, Division of Research Services, NIH
Department of Immunology, Litton Bionetics, Inc., Kensington, Maryland 20814

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Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The hypothesis that natural killer (NK) cells play an important role in immune surveillance against tumors has been based on data obtained in mouse experiments in which the results could have been influenced by immunological differences other than those observed in NK activity. There is therefore an obvious need for an in vivo model where animals, without manipulation, differ in NK activity only. The mutant beige mouse, which is NK-deficient, seemed promising until an additional T-cell defect was found. We have investigated the effects on several cellular immune functions of the transfer of nude genes to beige mice. The resulting viable, double homozygous recessive bg/bg nu/nu (beige-nude) mice combine the relative absence of T-cell function in regular nude mice, known to have high NK levels with the very low NK activity of beige mice. Therefore, such double immune deficient mice provide new possibilities for studying immune surveillance, particularly for establishing to what extent NK cells take part in host defense against spontaneous, induced, or transplantable tumors. Interestingly, no spontaneous malignant tumors have so far been observed in beige-nude mice.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel:

Øystein Fodstad (Co-Principal Investigator), Norsk Hydro's Institute for Cancer Research, Oslo, Norway

Carl T. Hansen, Veterinary Resources Branch, Division of Research Services, NIH, Bethesda, Maryland

Grace B. Cannon, Department of Immunology, Litton Bionetics, Inc., Kensington, Maryland

Other Personnel:

Mary G. McMenamin and Charles J. Sanders, Jr., Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

Athymic, nude mice are known to be deficient in functional T-lymphocytes. The low incidence of spontaneous tumors in such mice has drawn attention to thymus-independent, "non-specific" cellular mechanisms thought to be involved in destruction and growth inhibition of tumor cells in vivo. This natural cellular immune system includes natural killer (NK) cells, macrophages, polymorphonuclear leukocytes, and possibly K-cells and mast cells. The NK-cells are postulated to represent a primary line of defense. The importance of NK cells is supported by experiments where the growth in mice of NK-sensitive or resistant cells has been correlated to the level of NK activity. Differences in NK function in such studies has been due to differences in age, genotype or treatment with agents known to activate or suppress NK activity (e.g., Hanna and Fidler, JNCI 65: 801-809, 1980; 66: 1183-1186, 1981; Gorelik and Herberman, Int. J. Cancer 27: 709-720, 1981). However, the selectivity of the NK-cell impairment may be questioned, as other immune functions have been rarely investigated.

The in vivo importance of NK cells was seemingly demonstrated by enhanced growth of NK-sensitive tumor cells in mutant bg/bg mice with low NK levels compared to that in bg/+. However, it was later discovered that bg/bg mice also had a defective T-cell response (Talmadge et al., Nature 284: 622-624, 1980; Kärre et al., Nature 284: 624-626, 1980. The problem caused by the altered T-cell response in bg/bg mice could, however, possibly be circumvented. If one could breed athymic, T-cell deficient bg/bg mice that would retain a low NK activity, tumor growth control in such mice could be compared with that of regular T-deficient, nude mice with high NK levels. Preliminary work on beige-nude mice has been published by others, but more detailed studies are lacking, probably due to problems in viability of such double immune deficient mice. We therefore sought to examine in detail the immune characteristics of mice bred to have multiple immune defects.

Methods Employed:

By successive crossings and backcrossings between nu/nu mice on N:NIH(S) background and C57BL bg/bg mice we have succeeded in producing healthy non-inbred beige-nude mice. These animals showed an initially slower growth than that of regular nude mice. All mice were kept in specific pathogen-free (SPF) facilities until they reached a body weight of 15-20 grams (at 5-8 weeks of age), when they were transferred to conventional facilities and used within a few days for immunological studies. In this environment beige-nude mice used for other purposes had a life span of 6-10 months, similar to that of regular nude mice, whereas beige nude mice kept in SPF facilities lived for more than one year. This prolonged survival may at least partly be due to the increased robustness of non-inbred compared to inbred mice.

The effects of the introduction of nude genes on immune functions of mice was followed by testing spleen cells from beige and N:NIH(S) mice of the +/+, nu/+ and nu/nu genotypes.

All tests were performed on cells from individual animals, since assays using pooled spleen cells may not represent the mean of activities of individual cell populations. The experiments were repeated several times, always including 1-2 N:NIH(S) nu/nu and 1 Balb/c +/+ mice for reference.

Major Findings:

NK activity in vitro was determined for spleen cells from mice of the beige +/+, nu/+, nu/nu and NIH nu/nu genotypes. The cytotoxicity was measured for spleen cells for the three effector/target (YAC-1) ratios 50:1, 100:1 and 200:1. The level of NK activity in bg/bg mice was, as expected, very low. For beige mice, introduction of one nude allelic gene gave only a marginally increased NK activity, whereas two genes resulted in a small, but significant increase. Thus, the mean NK cytotoxicity (effector/target; 200:1) increased from 2% in bg/bg +/+ to 6% in bg/bg nu/nu. For NIH mice, the nude genes resulted in a considerable increase in NK function, and, most importantly, the difference in NK cytotoxicity between beige-nude (6%) and NIH nude (25%) mice was striking. The mouse to mouse variation in the NIH nu/nu group was more pronounced than in the other groups, in which, however, a similar variation would be difficult to detect due to the overall low values.

NK function is tested with a given ratio between dispersed spleen cells and target cells. Therefore, observed differences between mice in NK cytotoxicity could possibly be attributed to differences in relative numbers of NK cells versus other spleen cell types. This possibility could be ruled out, however, since the differences in NK levels between beige and NIH nude mice were independent of the total number of spleen cells.

The possible usefulness of beige-nude mice for testing the in vivo role of NK-cells in immune surveillance is dependent on whether differences in other cellular immune functions between beige and NIH nude mice could possibly exist. This was examined in several other assays.

The data confirmed that bg/bg +/+ mice have a defective T-cell response. The proliferative response to PHA and Con A was low compared to NIH +/+ (and Balb/c +/+) mice. Except for slightly lower values for beige homozygous nude mice, a similar difference could not be seen for the nu/+ and nu/nu genotypes. It should be noted that the nude mice were not completely devoid of cells responding to the T-mitogens.

Beige mice showed a low LPS (bacterial lipopolysaccharide) response compared to corresponding NIH and Balb/c mice. The differences in values were statistically significant and altogether the data provide strong evidence for the existence of a B cell defect linked to the beige genes. This was further supported by studies where the response to LPS was compared in C57 BL +/+, bg/+ and bg/bg mice. The introduction of nude allelic genes into NIH and beige mice was found to increase the response to the B mitogen LPS to very high levels. The response to LPS in nude mice has been regarded as normal and this effect of the nude genes has, to our knowledge, not previously been reported. Whether the increased LPS response can be explained, at least partly, by a relatively pure B cell population in spleens of nude mice, remains unknown. Interestingly, an apparent correlation is seen between the LPS and NK results for the different mouse groups.

In the plaque-forming cell assay, used to examine T-dependent antibody response to sheep red blood cells (SRBC), the lowest values were found for nu/nu mice. However, it is surprising that the functional capacity and cooperation between macrophages (accesory cells), T-cells and B-cells thought to be reflected in this test, is retained so well in T-deficient nude mice.

The NK cytotoxicity for the effector/target ratio of 200:1 in NIH and beige nu/nu were 25 + 2.8% and 6 + 1.1%, respectively. For these two genotypes the K-cell function was tested in an antibody-dependent cellular cytotoxicity (ADCC) assay, using Chang cells as targets. The results did correlate with the NK data for these mice, and hence do not contradict the suggestions by others that a relationship may exist between the effector cells in these two assays.

Significance to Biomedical Research and the Program of the Institute:

The comparison of immunological characteristics for beige and regular nu/nu mice suggests a promising role for the parallel use of these mice in studies on NK function in vivo, particularly in instances where rapidly acting immune mechanisms are thought to be of special or overriding importance. Host defense against spontaneously appearing malignantly transformed cells would be one such situation. It is interesting that whereas an increased incidence of spontaneous lymphomas in bg/bg +/+ mice has been reported, no spontaneous malignant tumor has so far been observed among our beige u/nu mice, even in animals living up to more than one year in SPF facilities. However, in 5-10% of beige-nude mice challenged i.v. or s.c. with poorly growing human tumor cells, a transient enlargement of lymph nodes and spleen was seen 3-5 weeks after tumor inoculation. Histology showed hyperplastic lymphoid tissue, and

attempts to transfer cells from these "tumors" to other nude mice, or to grow them in vitro, all failed. Thus, this seems to represent a benign lymphoproliferative reaction, probably in response to the tumor cells.

Proposed Course:

The important question whether the observed differences in immune function between beige and regular nude mice do result in a different ability to control tumor growth and metastasis formation of murine and human tumor cells, is now being investigated. Preliminary data does not seem to support the view that NK cells play an important role in host defense against tumors, but this possibility will be examined critically and carefully in our future studies, as it could have major impact on current views concerning the role of NK-cells in tumor defense.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07145-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathology of BCNU-induced lung injury

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Hildegard M. Reznik-Schuller, Visiting Scientist and Acting Head, Pathology and Ultrastructural Oncology Section, LETM, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Histopathological and electron microscopic studies of lung lesions induced in F344 rats by chronic BCNU treatment showed that the animals developed a severe interstitial fibrosis, emphysema, atelectasis, chronic bronchitis and peribronchitis as well as pneumonia. Macroscopically visible nodular lesions were identified as proliferated serous cells of peribronchial submucous glands accompanied by fibrosis and inflammation. Preneoplastic lesions (bronchial and bronchiolar hyperplasia, early squamous metaplasia, adenoma of peribronchial submucous gland and squamous metaplasia of proliferated submucous gland) were also found.

PHS 6040 (Rev. 1/83)

GPO 895-100

Other Professional Personnel:

Adaline C. Smith and Michael R. Boyd, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Anthony A. del Campo, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

The purpose of this project is to study the pathology of BCNU-induced lung lesions at the light and electron microscopic level.

Methods Employed:

The histopathology of lung lesions induced by BCNU was studied in three different groups of male F344 rats. Group 1 had been given BCNU in 6 doses at weekly intervals (individual dose: 5 mg/kg; total dose: 30 mg/kg). Group 2 had been given BCNU at the same dose level but at biweekly intervals. All dosed rats as well as 2 groups of vehicle controls were sacrificed 13 weeks after the last injection. The lungs were fixed with 10% buffered formaline and each lobe was embedded separately in paraffin. Electron microscopy was done on 5 of a third group of BCNU treated and one control rat. The dosed rats had been given 5 mg/kg BCNU i.p. once a week for 6 weeks and were killed 16 weeks after the start of treatment. Under pentobarbital anesthesia, tissue samples of macroscopically visible lung lesions were excised and processed for routine transmission electron microscopy (double fixation with cacodylate buffered glutaraldehyde and osmium tetroxide, embedding in Epon, uranyl acetate and lead citrate staining of thin sections).

Major Findings:

Macroscopically visible white, nodular lesions were found in the lungs of 22.2% of the animals in group 1, in 42% of group 2 but in no control animals. Histopathology revealed that these lesions were associated with bronchi and bronchioles. They consisted of an admixture of epithelial cells and fibrotic connective tissue elements accompanied by round cell infiltrates and occasional leukocyte infiltration. The lesions were always localized in the peribronchiolar (or peribronchial) tissue and contained proliferated acini of submucous glands. In addition to the lesions, emphysema, atelectasis and interstial fibrosis were found in all BCNU treated animals. Areas of emphysema and atelectasis were occasionally also found in the controls. Focal epithelial hyperplasias of bronchi or bronchioles were found in 22% of the group 1 animals, while in group 2, 58% of the rats demonstrated this type of lesion. In the latter group, 50% of the hyperplastic foci exhibited squamous metaplasia, while this change was not found in group 1. Moreover, in group 2, 25% of the animals demonstrated focal squamous metaplasia in peribronchial or peribronchiolar submucous glands and 1 animal had an adenoma arising from a peribronchial submucous gland. Hyperplasias and metaplasias of bronchi or submucous glands

was not found in any of the controls. In all of the BCNU-treated animals, the described lesions were accompanied by severe inflammations: chronic bronchitis and peribronchitis as well as pneumonia. In one of the control animals, peribronchitis and pneumonia were also found.

Electron microscopy of the peribronchial nodular lesions in group 3 revealed that the epithelial elements were derived from serous cells of the peribronchial submucous glands. These cells were characterized by a well developed rough endoplasmic reticulum and the presence of electron-dense round secretion granules. They tended to form acinar structures with a central lumen lined by scanty microvilli. In hyperplastic foci of bronchi and bronchioles, many of the epithelial cells were at an early stage of squamous metaplasia.

Significance to Biomedical Research and the Program of the Institute:

This is the first detailed pathological description of BCNU-induced lung lesions in an animal model. In view of the wide use of BCNU as an anticancer drug, these data are of obvious importance. Moreover, the pathology data compliment investigations on serum angiotensin converting enzyme activity and glutathione reductase done by other investigators of the LETM in the same animals.

Proposed Course:

The data obtained in this study do not yet allow for conclusions on the pathogenesis and mechanisms of induction of BCNU induced lung lesions. Such knowledge is, however, under way to develop methods for the prevention of the adverse side-effects of BCNU-treatment in the lungs. A serial sacrifice experiment will be conducted in which the development of BCNU-induced lung lesions will be studied by light and electron microscopy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07146-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Collaborative electron microscopy studies

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Various DTP Senior Staff; reported by A. A. del Campo, Biologist, Laboratory of Experimental Therapeutics and Metabolism, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.2	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Projects which have been under way include the following: A. Methods to produce consistent preparations of liposome-entrapped Melphalan: Liposomes or phospholipid vesicles have been used experimentally as a biodegradable carrier of cytotoxic drugs. Previous studies had shown that S.C. injection of liposomes containing Melphalan (MPL), a cytotoxic drug effective against mammary cancer, may be useful in treating lymph node metastases because lymphatic capillaries preferentially absorb fatty substances. A technique was developed in which the optical density of the liposome preparation was monitored as a function of sonication time and liposome size. The electron microscope was used to assess the morphology and size of the vesicles. Prolonged sonication produced liposomes that S.C. gave high and sustained concentration of MPL in lymph nodes of the rat; B. Mechanisms of in vitro tubulin polymerization induced by nucleotide analogs of GDP and GTP: Understanding the role of nucleotides in tubulin polymerization may aid in the design of effective cancer drugs since tubulin is a major component of the mitotic spindle which is intimately involved in cell division. The electron microscope was utilized to examine negative stains of tubules or thin sections of pelleted polymer material. These studies demonstrated that many nucleotide analogs of GDP and GTP supported polymerization; C. Ultrastructural characterization of malignant human cell lines maintained in vivo in athymic host systems. Our laboratory has established several human melanomas as xenografts in nude mice. Two of these melanomas (LOX and SESX) form metastases in the lungs of nude mice. This provided a means of investigating the mechanisms influencing lung colony formation. Samples from various sublines of LOX and SESX were examined in the electron microscope and several morphological features noted. Large amounts of intracytoplasmic filaments were particularly apparent in S.C. tumors of LOX and SESX. Cells of brain-passed tumors were observed as having numerous electron dense deposits within the cytoplasm. These and further observations may help explain the unusual metastatic behavior of these melanomas.

Other Professional Personnel:

Project A:

Juneji Khato and Susan Sieber, Laboratory of Medicinal Chemistry and Biology, NCI, Bethesda, Maryland

Project B:

Ernest Hamel and Chu M. Lin, Laboratory of Medicinal Chemistry and Biology, NCI, Bethesda, Maryland

Project C:

Øystein Fodstad, Norsk Hydro's Institute for Cancer Research, Oslo, Norway

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Other Personnel:

Anthony A. del Campo, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

Project A:

Melphalan (MPL) is a commonly utilized cytotoxic drug against a variety of tumors including mammary cancer, in which the regional lymph nodes are the common site of metastasis. However, it is difficult to remove all of the involved lymph nodes, especially those containing micrometastases, at the time the primary tumor is resected. Liposomes containing MPL may be useful in the therapy of lymph node metastasis because it is known that lymphatic capillaries absorb fatty substances.

Previous studies conducted by Dr. Khato (LMCB) on lymph node uptake of liposomes containing MPL revealed the importance of producing preparations of liposomes reproducibly with respect to size and drug entrapment rate. Small liposomes are usually prepared by sonication until the sonicate becomes "clear." However, the time required for clarification of a sonicate is not consistent because of several variables involving the sonicator. Therefore, it has been difficult to determine a suitable endpoint for sonication. Obviously, a more quantitative way to assess the duration of sonication is desirable. The present work evaluated optical density as an index to monitor sonication time so that more reproducible liposome preparations can be obtained.

Project B:

Guanosine nucleotides are intimately involved in the structure and function of tubulin, the major component of the mitotic spindle. Since understanding the

role of nucleotides in tubulin polymerization may aid in the rational design of effective antineoplastic agents, Dr. E. Hamel and coworkers (LMCB) have been studying the effects of GDP and GTP analogs. The LETM (Mr. del Campo) has collaborated in these studies using electron microscopy to evaluate the nature of the material formed after polymerization induced by nucleotide analogs of GDP and GTP.

Project C:

Dr. Fodstad has established several human melanomas as xenografts in nude mice. Two of the melanomas (LOX and SESX) grow in the lungs of adult nude mice upon intravenous injection. This ability provided a means of investigating the mechanisms influencing lung colony formation. To pursue this fully, it was necessary to conduct an ultrastructural evaluation of LOX and SESX melanomas to correlate morphological characteristics with biochemical data such as cytogenetics which indicated that sublines of the tumor had different chromosome numbers.

Methods Employed:

Project A:

Liposome suspensions were observed in the electron microscope to assess the morphology of the vesicles and make size measurements. A negative stain technique incorporating ammonium molybdate as the contrasting stain was utilized.

Project B:

Both negative stain and sectional material were examined in the electron microscope. In some cases, specimens were prepared utilizing a special EM technique involving fixation in tannic acid/glutaraldehyde and embedded in a high resolution epoxy resin. This permitted the subunit structure of the microtubules to be readily observed.

Project C:

Tumor samples from various sublines of the LOX and SESX melanoma growing in vivo were processed for electron microscopy using a routine method of fixation, dehydration and embedding. Ultrathin sections of these samples were obtained and examined in the electron microscope.

Major Findings:

Project A:

Electron microscopic examination of suspensions produced by prolonged sonication revealed vesicles that were small and round and relatively uniform in size and shape. The mean diameter of these vesicles was 36.9 ± 7.7 nm. Short sonication times (2-4 min) OD = 1.8-2.0 produced cloudy suspensions with higher entrapment rates for both MPL and the aqueous phase. These suspensions contained mostly large vesicles but were notably variable in size and shape.

Project B:

Deoxy GTP (dGTP) analogs and arabinosyl GTP all supported the polymerization reaction in which microtubules were formed in the presence of MAP's. However, the reaction began earlier and required a lower nucleotide concentration when compared to GTP-supported reaction. 2',3'-Dideoxyguanosine-5'-triphosphate (ddGTP) was unique among the analogs tested in that it supported polymerization without MAP's, although a higher nucleotide concentration was required. The polymer was a mixture of microtubules and open sheets. In addition, microtubules formed with ddGTP plus MAP's were significantly more cold stable than microtubules formed with GTP plus MAP's.

Analogs with an open ribose ring bearing a methyl or phosphate group at the 2' or 3' hydroxyl were also examined. 2',O-Methylguanosine-5-triphosphate (2',O-MeGTP) was comparable to GTP in its ability to support MAP dependent polymerization as a function of nucleotide concentration. Progressively decreasing activity was observed with acycloguanosine-5'-triphosphate (3',O-MeGTP) and the periodateoxidized, borohydride-reduced derivative of GTP (ox-redGTP). Microtubules were formed with acyclo GTP and 3',O-MeGTP, but thick-wall enlarged tubules about 80-82 nm in diameter were formed with 2',O-MeGTP and Ox-redGTP.

No definite protofilaments could be distinguished in these bizarre structures. If nucleodiphosphate kinase was included in the reaction mixture, microtubules became the predominate product with ox-redGTP.

The stability of the tubulin polymer was also studied and compared to the stability of microtubules formed with GTP. Our results have supported the conclusion that nucleotide triphosphate hydrolysis enhances the stability of microtubules. Polymers formed with ddGTP and MAPs (GTP hydrolysis is required for polymerization) were more cold-stable than either ddGTP and MAPs (GTP hydrolysis not required for polymerization) or GTP and MAPs. In fact, ddGTP formed microtubules at 0°C. The most striking difference in the stability of the two types of microtubules was obtained in the experiments with Ca^{2+}. Addition of $CaCl_2$ to polymer resulted in complete depolymerization of microtubules formed with either GTP or ddGTP and MAPs. However, the polymer formed with ddGTP with or without MAPs was stable to Ca^{2+}.

The dilutional stability of microtubules formed with the two nucleotides was essentially identical. Polymers formed in the presence of MAPs and GTP or ddGDP or ddGTP as well as ddGTP minus MAPs were all sensitive to a decrease in tubulin concentration. However, these experiments also showed that the critical concentration for tubulin with ddGTP was approximately halved by adding MAPs to the reaction. It was also observed that the polymer formed with ddGTP and MAPs was more cold-stable than without MAPs. Significant depolymerization occurred at 25°C without MAPs but only at 5°C with MAPs. Reaction mixtures containing MAPs and GTP or ddGTP in which phosphofructokinase were less labile to rapid triphosphate depletion when compared to mixtures without MAPs.

Microtubules formed with ddGDP and MAPs were unaffected by phosphofructokinase addition. These experiments all emphasized the importance of MAPs in stabilizing tubulin polymers.

Project C:

The most striking feature in the melanomas was the presence of large amounts of intracytoplasmic filaments, particularly apparent in the subcutaneous tumors of LOX, LOX-N and SESX. These filaments measured 60-80°A in diameter and were usually observed in bundles, often situated near the periphery of the cell. This was an interesting finding since cytoplasmic filaments are not generally noted as an ultrastructural feature of melanomas. This abundance of filaments might represent an increased level of cell motility which could help explain the unusual metastatic behavior of these tumors.

Brain-passed tumors, on the other hand, did not exhibit the same marked accumulation of filaments. These cells were observed as having numerous electron dense deposits within the cytoplasm. These deposits did not appear to possess the characteristic internal structure of melanomas and may represent some cell product whose synthesis may be initiated once transplanted in the brain. Electron dense deposits were also frequently seen in LOX-N-I.V.-lung and SES-S.C., but were not observed in LOX-S.C. and LOX-N-S.C.

Significance to Biomedical Research and the Program of the Institute:

Ultrastructural evaluation provides an important adjunct to studies of mechanisms of antitumor drug action and toxicity. It also is an important tool necessary to probe the structural/physiological/biochemical correlations essential for tumor biology investigations.

Proposed Course:

Projects A and B are essentially complete and no further studies are planned. Project C is nearing completion, and it is anticipated that evaluation of the remaining samples on hand will be completed soon.

Publications:

1. Khato, J., del Campo, A.A. and Sieber, S.M.: Carrier activity of sonicated small liposomes containing Melphalan to regional lymph nodes of rats. Pharmacology 26: 230-240, 1983.

2. Hamel, E., del Campo, A.A., Lustboder, J., and Lin, C.M.: Modulation of tubulin-nucleotide characterizations by microtubule-associated proteins: Polymerization with ribose-modified analogues of guanosine 5'-triphosphate. Biochemistry 22: 1271-1279, 1983.

3. Hamel, E., del Campo, A.A. and Lin, C.M.: Microtubule assembly with the GDP analog 2',3'-dideoxyguanosine 5'-diphosphate. Biochemistry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07147-01 LETM

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pathology of doxorubicin-perfused lungs of dogs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation) Hildegard M. Reznik-Schuller, Visiting Scientist and Acting Head, Pathology and Ultrastructural Oncology Section, LETM, NCI

COOPERATING UNITS *(if any)*

Surgery Branch, Clinical Oncology Program, Division of Cancer Institute, National Cancer Institute

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The histopathology of Doxorubicin perfused lungs of dogs was studied in animals perfused with different concentrations of the drug. The acute effects were severe perivascular and subpleural edema and emphysema. Up to 4 weeks after the start of treatment, pneumonia, pleuritis, atelectasis and early interstitial fibrosis developed from such lesions.

Other Professional Personnel:

Michael R. Boyd, Chief, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Rodney F. Minchin, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Michael R. Johnston, Surgery Branch, NCI, Bethesda, Maryland

Other Personnel:

Marybelle Gregg, Laboratory of Experimental Therapeutics and Metabolism, NCI, Bethesda, Maryland

Objectives:

The purpose of this project is to study the pathology of lung lesions induced by Doxorubicin perfusion of the lungs in dogs.

Methods Employed:

The histopathology of lung lesions induced by Doxorubicin perfusion of the left lungs was studied in 6 dogs. The experimental design was as follows: the dogs' left lungs were perfused with different concentrations of Doxorubicin and a pneumonectomy was done on the untreated right lungs 2 weeks later. Four weeks after the start of the experiment all surviving animals were sacrificed. The concentrations of Doxorubicin used were: 15 µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml. Histopathology from formalin fixed, paraffin embedded, and hematoxylin/eosin stained tissues was conducted from preperfusion biopsies, postperfusion biopsies, the right pneumonectomized lungs and representative samples of each lobe of the left lungs at sacrifice.

Major Findings:

The high dose animals died within 24 hrs postperfusion while some of the middle dose and all of the low animals survived until sacrifice.

The acute effects were severe perivascular and subpleural edema and acute emphysema of the lungs. From such changes, interstitial pneumonias accompanied by beginning fibrosis and atelectasis, as well as adhesive pleuritis developed in the animals that survived longer.

Significance to Biomedical Research and the Program of the Institute:

These investigations are part of a large interdisciplinary program to establish a lung perfusion system for therapy of metastatic and primary tumors in the human lungs. If the system works successfully, this would eliminate adverse side-effects of the drug in other organs. The importance for lung cancer therapy in general is obvious. The pathology studies serve as a basis for evaluating toxic effects and to find doses that are tolerated by the patients.

They also broaden our knowledge on the mechanism of action of the drug in the lungs.

Proposed Course:

The pathology studies will continue to help monitor on an objective basis the adverse effects of future perfusion experiments. Moreover, electron microscopy will be applied in low dose groups to elucidate the intracellular changes and target cell types of the drug. Electron microscopy will also be applied to human patients undergoing perfusion therapy in order to establish correlations between type and differentiation of the lung tumors and the effects of the therapy.

ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 - September 30, 1983

The Laboratory of Medicinal Chemistry and Biology was established in 1975 in order to provide a facility capable of antitumor drug development from the stage of design and synthesis through biochemical and pharmacological characterization to Phase I clinical trial. The original organizational components were the Drug Design and Chemistry Section, concerned with the rational design and synthesis of new agents, and the Applied Pharmacology Section, concerned primarily with fundamental mode of action studies, but also participating in biochemical studies on material derived from Phase I clinical trials of new antitumor agents. The Office of the Chief, LMCB, in addition to assuming administrative responsibilites for the Laboratory as a whole, also maintained an active research interest in the mode of action of new agents and the identification of new sites for pharmacological attack. In 1980, the Laboratory underwent a major expansion, with the addition of a Biochemistry Section, concerned with the mechanisms by which antitumor agents exert their inhibitory effects on purine biosynthetic pathways, and a Drug Interactions Section, concerned primarily with the role of drug metabolism in drug toxicity and therapeutic activity.

Within the Drug Design and Chemistry Section, work has continued during the past year both on the synthesis of new drugs which will be clinically useful in the treatment of cancer, and/or the development of analytical support for pharmacokinetic studies of agents which originated within the Section, and which are now in Phase I/II clinical trial.

Several avenues are being pursued in the design and synthesis of new agents. Last year, we reported the synthesis of the fraudulent NAD analogue adenine-D-ribose-phosphate-phosphate-D-ribose-thiazole-4-carboxamide (TAD), the active metabolite of the antitumor agent tiazofurin (NSC-286193). This anabolite acts by inhibiting the enzyme IMP dehydrogenase, a key step in the purine biosynthetic pathway. Over the past year, six analogues of TAD have been synthesized, and the biological activity of three of these has been evaluated. Of particular interest is the analogue adenine-D-ribose-phosphate-phosphate-D-ribose-selenazole-4-carboxamide, a compound several-fold more active than TAD as an IMP-dehydrogenase inhibitor.

Another topic of particular interest in the synthetic area is the preparation of transition-state inhibitors of the enzyme purine nucleoside phosphorylase (PNP-ase). Inhibitors of this enzyme are of interest because of their potential application in perturbing endogenous purine nucleotide pools, and also in potentiating the antitumor activity of purine nucleoside analogues in vivo. The key intermediate has been successfully synthesized and the preparation of this interesting series of inhibitors is now in progress, with biological activity being assessed within the Drug Metabolism Section of the Laboratory. This intermediate is also of potential value in the total synthesis of the

nucleoside analogue neplanocin, an active antitumor agent hitherto obtained only as a natural product.

The development of new analytical techniques for agents of interest to LMCB has continued. The objectives of these studies are to establish the structure and purity of new antitumor agents, and also to elucidate reaction mechanisms and to develop assays for the quantitation of these agents in physiological samples. Mass spectrometry, gas-liquid chromatography and HPLC techniques are used. Extensive clinical pharmacology studies have been continued with LMCB-developed agents now in Phase I/II clinical trial (AZQ and DHAC), and the major pharmacokinetic parameters for these two compounds have been elucidated. Extensive studies have been carried out with the differentiation-inducing agent HMBA (hexamethylene bis-acetamide). Experiments have been undertaken in rats, in collaboration with the Drug Interactions Section (Dr. C. Litterst), to determine the feasibility of maintaining HMBA concentrations in vivo which match exposure conditions in vitro. A rapid and simple gas chromatographic method incorporating an internal standard and involving the direct analysis of plasma or urine has been developed for the measurement of HMBA in these fluids. A limit of detection of less than 50 μg/ml is obtained and the small amount of plasma required for sample workup permits multiple sampling from treated animals. A single iv bolus dose of 800 mg/kg HMBA results in plasma levels well above 5 mM, but the compound is rapidly eliminated from the plasma ($t_{1/2}(\alpha)$=5.5 min; $t_{1/2}(\beta)$ =122 min) with a total body clearance of 7 ml/min/kg. The primary route of excretion appears to be urinary since more than 65% of the administered dose is found as unchanged HMBA in the urine after the first 8 hr. Future work will be directed towards determining what HMBA plasma levels can be maintained through continuous infusion of non-toxic doses in unanesthetized animals.

At the molecular level, studies have been initiated in the Applied Pharmacology Section on the 5-azacytidine analogue arabinosyl-5-azacytosine (araAC; NSC-281272), a compound recently developed within the Drug Design and Chemistry Section. AraAC has murine antitumor activity comparable to that of 5-azacytidine and to arabinosylcytosine (araC); possibly more important, however, is its activity in vivo against the mammary, colon and lung human tumor xenografts as compared to 5-AC (inactive), 2'-deoxy-5-AC (inactive) and DHAC (active against the mammary tumor only). Studies are currently underway within the Applied Pharmacology Section comparing araAC, araC, DHAC, 2'-deoxy-5-AC and 5-AC with respect to their cytotoxicity, metabolism, incorporation into DNA and RNA and effects on synthesis and methylation of DNA. Since araAC is more active than the other analogues against the CX-1 human colon tumor xenograft, the tissue culture version of this tumor (HT-29 cells) will be particulary useful in assessing the mode of action of this compound.

Within the Biochemistry Section, studies have continued on the mode of action of tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide; NSC-286193), a compound which has recently entered NCI-sponsored Phase I clinical trial. In studies of a spectrum of 6 murine and 6 human tumor cell lines, with varying degrees of sensitivity to the drug, it was established that an excellent correlation exists between the ability of a tumor line to convert tiazofurin to its active anabolite TAD (see above), and the cytotoxic effects of the drug. In related studies, the factors responsible for the greater antitumor activity of the corresponding selenium analogue selenazofurin, as compared to the parent sulfur compound,

tiazofurin, have been explored. It has been established that selenazofurin, like tiazfurin, undergoes anabolism to an analogue of NAD, and that the latter is also a potent inhibitor of IMP dehydrogenase. The greater in vivo activity of selenazofurin appears to result from two factors: i. the fractional conversion to the NAD analogue is greater for the selenium compound than for the sulfur compound; and ii. the selenium analogue of NAD has a higher affinity for IMP dehydrogenase than does the sulfur analogue (TAD). These results will be of direct clinical relevance should the selenium compound be selected for therapeutic trial in man.

In the Drug Interactions Section, studies have been conducted over a period of years on the molecular toxicology of cisplatinum (cisplatin; cisPt) and other platinum containing antitumor drugs. A definitive study of the renal handling of cisPt has now been completed. The renal clearance of free cisplatin was the same as the glomerular filtration rate (GFR). Probenecid resulted in a 70% increase in GFR but N-methylnicotinamide did not affect GFR. Cisplatin is excreted by a process which includes active reabsorption via the organic acid transport system. Ammonium chloride (AC)-pretreated rats did not exhibit the characteristic cisplatin induced diuresis and were unable to maintain an acidic urinary pH. AC-pretreated rats had a lower, and sodium bicarbonate (SB)-pretreated rats a higher, urinary excretion of Pt than did controls. Platinum excretion was correlated with urinary pH and not urinary volume. The renal concentration of platinum was greater in AC animals than in SB or TW animals. Both pretreated groups had equal percent of free vs. bound platinum. Proteinuria was more severe in AC-pretreated rats, but histologic evidence of tubular damage was present in all groups. Enhanced Pt concentrations were observed in ovaries and uterus after cisplatin was administered into the common iliac artery. A therapeutic advantage may be gained by regional IA administration of cisPt for pelvic neoplasms. These studies, together with ancillary pharmacokinetic studies, are now being extended to the platinum analogue CBDCA, presently in early clinical trial.

Within the Office of the Chief, studies have continued on antineoplastic agents which act on tubulin, a protein critical for cell division. We have recently found that an analog of GTP modified in the 5'-triphosphate moiety [guanosine-5'-O-(3-thiotriphosphate)] is a potent inhibitor of tubulin polymerization and tubulin-dependent GTP hydrolysis. An improved preparation of microtubule associated proteins is being developed to obtain these proteins in large amounts. We have also found that a new plant-derived antineoplastic agent, combretastatin (NSC-348103), has both antimitotic and antitubulin activity, and its mechanism of action is being examined. Based on earlier studies from this laboratory which demonstrated conditions in which tubulin-dependent GTP hydrolysis was totally dependent on taxol (NSC-125973), we were able to develop an assay for the drug sensitive to 0.1 μM. Conditions were established suitable for measuring serum concentrations, and a preliminary pharmacokinetic study has been performed in rabbits. Although taxol appears to be protein-bound in serum, it is rapidly cleared with α-phase and β-phase half-lives of 2.7 and 42 min, respectively. This assay is suitable for human pharmacokinetic studies, and has been made available to clinical research groups conducting Phase I/II trials with this agent.

Efforts have also continued within the Office of the Chief, to elucidate the mode(s) of resistance to the clinically important antitumor agent melphalan (L-

PAM). Murine L1210 leukemia cells resistant to L-PAM have been sensitized in vitro and in vivo by reducing the cellular concentration of the tripeptide glutathione. Such reduction can be achieved in vivo by implementation of either nutritional or pharmacological regimens. Nutritional reduction of tumor cell glutathione is carried out by short term (1 week) maintenance of host animals on defined amino acid diets devoid of L-cystine and with L-methionine reduced to 25% of control. These results indicate a preferential reduction of tumor cell glutathione and selective sensitization of the resistant tumor cell to L-PAM when compared to host bone marrow progenitor cells. Pharmacological reduction of tumor cell glutathione can be achieved by constant infusion, via osmotic pumps, of D,L-buthionine-S, R-sulfoximine, an inhibitor of glutathione biosynthesis, into tumor-bearing mice. The therapeutic implications of these observations in L-PAM sensitive and resistant human ovarian tumor cell lines are now being explored in collaboration with Medicine Branch, COP.

The preceding outline summarizes the objectives of the Laboratory of Medicinal Chemistry and Biology, and describes some of the research carried out within the Laboratory during the year. The bibliography for the Laboratory as a whole is listed below, followed by the individual Project Reports which describe this research in greater detail.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CM07102-08 LMCB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tubulin Structure and Microtubule Formation as Sites for Pharmacologic Attack

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Ernest Hamel, M.D., Ph.D. Cancer Expert LMCB NCI

COOPERATING UNITS *(if any)*
Laboratory of Experimental Therapeutics and Metabolism, DCT, NCI

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Office of the Chief

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.0	1.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interactions between the polypeptide subunits of tubulin and its two tightly bound guanine nucleotides. Interactions of ribose-modified GDP and GTP analogs with tubulin were examined in a microtubule-associated protein-dependent polymerization system. Inhibitory effects correlated well with nucleotide affinity for the exchangeable site, but the ability of analogs to support polymerization had no relationship to their affinity for tubulin. Microtubules formed with nucleotide hydrolysis were more stable than those formed without hydrolysis. The analog guanosine 5'-O-(3-thiotriphosphate) was a potent inhibitor of tubulin polymerization and GTP hydrolysis. Improved methods for the preparation of microtubule-associated proteins were developed. A new plant-derived antimitotic drug with antitubulin activity, combretastatin, was described. Efforts to separate the α and β subunits of tubulin continued.

Microtubules, cellular organelles critical for cell division, are sensitive to a number of antineoplastic drugs. Their major constituent is an acidic protein known as tubulin, which consists of two different polypeptide chains and two molar equivalents of GTP. Half this GTP (the exchangeable nucleotide) is hydrolyzed during microtubule assembly from tubulin and microtubule-associated proteins (MAPs -- minor, but essential, components of the microtubule). Studies with ribose-modified GDP and GTP analogs were continued to explore nucleotide structural requirements at the exchangeable site. All modifications of the ribose moiety resulted in substantial reductions of nucleotide affinity for tubulin; but a notable disjunction between the affinity of a GTP analog for the exchangeable site and its ability to support polymerization was observed. Inhibitory effects of GDP analogs on tubulin polymerization, however, were in agreement with their affinity for tubulin. These studies also demonstrated that nucleotide hydrolysis resulted in significant enhancement of the stability of microtubules. We have recently found that an analog of GTP modified in the 5'-triphosphate moiety [guanosine-5'-O-(3-thiotriphosphate)] is a potent inhibitor of tubulin polymerization and tubulin-dependent GTP hydrolysis. These effects will be studied in detail. An improved preparation of MAPs is being developed to obtain these proteins in large amounts. We have also found that a new plant-derived antineoplastic agent, combretastatin (NSC-348103), has both antimitotic and antitubulin activity, and its mechanism of action is being examined. We have found that tubulin-nucleotide interactions are profoundly affected by divalent cation concentration, tubulin concentration and pH. These effects are being studied in detail. Finally, we are continuing to attempt the preparative separation of the two subunits of tubulin and reconstitution of the protein's activity from its subunits.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM07104-08 LMCB

PERIOD COVERED

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TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

L-Phenylalanine Mustard Cytotoxicity and Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

David T. Vistica, Ph.D., Pharmacologist, LMCB

COOPERATING UNITS *(if any)*

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Office of the Chief

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Murine L1210 leukemia cells resistant to L-phenylalanine mustard (L-PAM) can be sensitized in vitro and in vivo to L-PAM by reducing the cellular concentration of the tripeptide glutathione. Such reduction can be accomplished in vivo by implementation of either nutritional or pharmacological regimens. Nutritional reduction of tumor cell glutathione is accomplished by short term (1 week) maintenance of host animals on defined amino acid diets devoid of L-cystine and with L-methionine reduced to 25% of control. These results indicate a preferential reduction of tumor cell glutathione and selective sensitization of the resistant tumor cell to L-PAM when compared to host bone marrow progenitor cells. Pharmacological reduction of tumor cell glutathione can be achieved by constant infusion, from alzet mini-osmotic pumps, of D,L-buthionine-S, R-sulfoximine, an inhibitor of glutathione biosynthesis, into tumor-bearing mice.

Objective: This project is designed to determine whether glutathione-mediated cellular resistance to selected alkylating agents can be reversed by employing nutritional and pharmacological regimens designed to reduce tumor cell glutathione.

Methods Employed: A. Nutritional reduction of Glutathione. Male CDF_1 mice were placed on specific diets for a period of 1 week prior to inoculation with L-PAM resistant tumor cells. Tumor cells were removed 3 days following inoculation into mice on diets and assayed for glutathione content and sensitivity to L-PAM in vitro. Bone marrow cells were removed by aspiration from femurs of mice on the same diets for 1 week, red cells removed by hypertonic lysis in 0.87% NH_4Cl and the glutathione content and sensitivity to L-PAM determined. B. Pharmacological reduction in cellular glutathione. Alzet mini-osmotic pumps containing DL-buthionine S, R-sulfoximine were implanted intraperitoneally into 28-30g male CDF_1 mice. The mice were inoculated with the L-PAM resistant tumor 24 hours later and treated with L-PAM 24 hours following tumor inoculation.

Major Findings: A. Nutritional Reduction of Glutathione In Vivo. The content of glutathione of L-PAM resistant tumor cells isolated from mice on a defined amino acid diet devoid of L-cystine and with L-methionine reduced to 25% of control is reduced more than 2-fold as compared to a 25% reduction in the glutathione content of bone marrow cells. Reduction in the glutathione content of the resistant tumor cell is accompanied by sensitization to L-PAM while the observed reduction in the glutathione content of bone marrow cells resulted in no sensitization of the progenitor cell to L-PAM. B. Pharmacological Reduction of Glutathione. Single injections of DL-buthionine S,R sulfoximine were ineffective in vivo in reducing cellular glutathione content of the resistant tumor cell. However, constant infusion of the inhibitor of glutathione biosynthesis from Alzet mini-osmotic pumps reduced cellular glutathione and sensitized resistant tumor cells confined to the peritoneal cavity to L-PAM.

Publications

1. Vistica, D.T.: Cellular pharmacokinetics of the phenylalanine mustards. Pharmacol. Ther., in press.

2. Suzukake, K., Vistica, B.P. and Vistica, D.T.: Dechlorination of L-phenylalanine mustard by sensitive and resistant tumor cells and its relationship to intracellular glutathione content. Biochem. Pharmacol. 32: 165-167, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM07109-07 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Anticancer Drugs on Cell Viability and the Synthesis of Nucleic Acids

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Robert I. Glazer, Head, Applied Pharmacology Section, LMCB, DTP, DCT, NCI

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SECTION

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.5	4.5	1.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The action of anticancer drugs on the synthesis, methylation and function of nucleic acids will be assessed in human tumor cell lines in culture. The nucleoside analogs, 5-fluorouridine, 5-azacytidine, sangivamycin, formycin, 8-azaadenosine and thiazolecarboxamide riboside, the anthracyclines, 3'-deamino-3'-morpholinodaunorubicin and 5-iminodaunorubicin and human interferons α, β, and γ will be examined. Analyses of the synthesis and processing of ribosomal precursor RNA and messenger RNA, as well as the synthesis, methylation and integrity of DNA will be conducted. 2',5'-Oligoadenylate synthesis and polyamine-activated protein phosphorylation will be assessed in human colon carcinoma cells treated with various types of interferon.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Investigators:

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Marvin B. Cohen, Ph.D.	Staff Fellow
Kathleen D. Hartman, B.S.	Chemist
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James B. Johnston, M.D.	Visiting Fellow
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1. Adenosine Analogs

Previous studies by this Section have shown that drugs such as cordycepin (3'-deoxyadenosine) and xylosyladenine were markedly potentiated by the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), but were weak inhibitors in vitro and in vivo when adenosine deaminase was not inhibited. Because of the limiting effects of adenosine analog deamination, there has been renewed interest in compounds which are inherently resistant to this catabolic effect such as the pyrrolopyrimidines typified by the parent drug in this class, tubercidin (7-deazaadenosine). We initially began cytotoxicity studies with another agent in this class, sangivamycin (7-deaza-7-carboxamidoadenosine), as a result of experiments with murine S-180 cells in culture which documented that sangivamycin produced a striking time-dependent toxicity. This effect was confirmed in the human colon carcinoma cell line HT-29 and appeared to be associated temporally with the incorporation of drug into total poly(A)RNA (mRNA). Subsequent studies established that either total mRNA or poly(A)RNA from sangivamycin-treated cells possessed reduced translational activity in a rabbit reticulocyte lysate system in vitro. Based on the experimental studies with this drug, a phase I clinical trial is being conducted by Dr. Paul S. Ritch at the Medical College of Wisconsin in collaboration with this Section.

The research aims for the studies with pyrrolopyrimidines will be to examine the role of protein synthesis in their cytotoxicity. Since this is a diverse family of drugs, we will attempt to determine if there is a structure activity relationship between the functionality at the C-7 position and their growth inhibitory effects, cytocidal activity and effects on nucleic acids and protein synthesis. It is apparent from past studies that the incorporation of sangivamycin into poly(A)RNA shows a positive correlation with its cytocidal activity in mouse and human tumor cells in vitro. This effect results in slight to moderate inhibition of mRNA translational activity in vitro (manuscript submitted for publication). Therefore, the consequences of this action on protein synthesis will be explored by measuring amino acid incorporation into protein, polyribosome integrity and initiation of translation in situ. Another aspect to this project will be to examine the relationship between the synthesis and processing of nucleolar RNA, protein synthesis and cell lethality with respect to the mechanism of action of these agents.

2. Pyrimidine Analogs

One of the primary interests of this Section over the past few years has been the mechanism of action of 5-substituted pyrimidine analogs such as 5-fluorouracil (5-FU), fluorouridine (5-FUR) and 5-azacytidine (AZC). We initially

documented that high concentrations of 5-FU could be incorporated into rRNA and mRNA in regenerating liver as well as L1210 ascites cells. Other investigators reported a strong association between the incorporation of 5-FU into total RNA and tumor cytotoxicity. These investigations have led to an analysis of the translational and posttranscriptional changes in various species of RNA following 5-FU, 5-FUR and AZC treatment. In vivo in the regenerating liver model, little change or slight increases in translational activity of certain peptides were observed with 5-FU-substituted mRNA using a wheat germ extract assay in vitro. No significant changes in translational activity were noted using 5-FU-substituted mRNA from Ehrlich ascites cells and similar results were found for AZC-modified mRNA from the same cell line. Subsequent experiments have failed to show any relationship between the translational ability of 5-FU-modified mRNA and cell lethality in human colon carcinoma cells. Other experiments established that polyadenylylation, as well as tRNA methylation were inhibited by 5-FU and AZC treatment. Cell culture studies also showed a positive correlation between sensitivity to 5-FU and incorporation of 5-FU and 5-FUR into nuclear RNA.

One area we propose to study involves measuring the processing of mRNA in ribonucleoprotein particles (RNPs) by the use of psoralen cross-linking in vivo. This procedure should give us a first hand picture of the ability of cells to splice mRNA via small nuclear RNA (snRNA) interactions with heterogeneous nuclear RNA precursors to mRNA. We also propose to study the effect of drug substitution in RNP's by measuring the translational activity of these particles in cell-free systems, as well as their protein composition. The former activity should be more relevant to in vivo cytotoxicity since mRNA in its native form is probably translated as an RNP. RNP's also have a characteristic composition dominated by a 40,000 dalton protein, and it should be informative to compare the protein composition of RNP's after drug treatment by one- and two-dimensional gel electrophoresis. Another aspect to this proposal would be to measure the levels of the uridylic acid-rich snRNA, U2 RNA, which is thought to be involved in mRNA splicing. The use of blot hybridization techniques with cloned DNA to internal coding sequences of U2 RNA should provide us with a sensitive means for quantitating changes in its synthesis and turnover in 5-FU- or AZC-treated cells.

Of particular interest to this Laboratory has been the development of AZC analogs with enhanced therapeutic efficacy. Arabinosyl-5-azacytosine (araAC) was developed by the Drug Design and Chemistry Section to meet this criterion. araAC has shown comparable antitumor activity to AZC as well as to arabinosylcytosine (araC). Even more important has been its enhanced activity in vivo against the mammary, colon and lung human tumor xenografts vs. AZC (inactive), deoxyAZC (inactive), DHAZC (active only against the mammary tumor). Current studies are underway comparing araAC, araC, deoxyAZC and AZC with respect to their cytotoxicity, metabolism and their incorporation into DNA and RNA and their effects on the synthesis and methylation of DNA. Since araAC is more active than the other analogs against the CX-1 human colon tumor xenograft, the tissue culture version of this tumor (HT-29 cells) should be particularly relevant for assessing their mechanism of action.

3. Benzoquinone- and Sugar-Modified Anthracyclines

Among the more interesting developments in the class of anthracycline antitumor agents have been the synthetic approaches by Dr. Edward M. Acton, Stanford Research Institute. In an attempt to increase the therapeutic index and efficacy of anthracyclines, his laboratory has synthesized analogs with mainly two modifications: 1) 5-imino substitution in the benzoquinone ring and 2) sugar amine substitutions. 5-iminodaunorubicin (IM) is an especially intriguing drug since it was the first anthracycline with a modified benzoquinone functionality and rendered the drug resistant to spontaneous or the indirect generation of free radicals. Our initial studies with 5-iminodaunorubicin (IM) indicated that it was equally or slightly less active than Adriamycin (ADR) against human colon carcinoma cells depending on the exposure interval. The cell lethality produced by IM was not related to inhibition of nuclear or total RNA synthesis, in contrast to the pronounced effect of ADR on nuclear RNA. Recent investigations of anthracycline-induced DNA-protein crosslink-associated single strand breaks revealed that IM was equally as effective as ADR in producing DNA scission and that this phenomenon was directly related to intracellular drug concentration. Those data, as well as recent investigations of the effect of anoxia on anthracycline-associated DNA scission suggests that the latter phenomenon is not related to generation of free radicals.

The second type of anthracycline modification has involved several heterocyclic substitutions of the sugar amine. In vivo studies against P388 leukemia demonstrated that morpholinyldaunorubicin (MD) and methoxypiperidinyl-daunorubicin (MEO) had enhanced efficacy with MD being 40-fold more potent than daunorubicin (DAU). Subsequent investigations with HT-29 cells by our laboratory revealed MD to be less potent after 2 hr treatment but more potent that the parent drug after 24 hr treatment. The latter differences vs. in vivo studies were explainable by the rapid metabolism and efflux of MD and the reduced efflux of MEO vs. ADR or DAU. The cytocidal activity of these analogs as well as the 13-dihydro alcohol metabolites were not related to inhibition of RNA or DNA synthesis, but the latter properties of these drugs were a function of their nuclear affinities for chromatin. Current studies are directed toward investigating cyanomorpholinyl ADR (CMA) which is 1000 times more potent than ADR in both P388 leukemia in vivo. Preliminary studies with this analog, which appears to be the most potent synthetic anticancer drug yet discovered, suggest that impaired transcription is related to cell lethality. CMA will be studied further for its chromatin binding to nuclei in HT-29 cells, as well as its in vitro effect on transcription in isolated nuclei from this tumor cell line.

4. Interferon and 2',5'-Oligoadenylate Analogs

One of the prominent biochemical features of cells treated with interferon (IFN) is the induction of the double-stranded RNA (dsRNA)-dependent enzyme, 2'5'-oligo (A) synthetase which results in the biosynthesis of ppp5'A(2'p5'A)n (2',5'-oligo (A)), an activator of a latent ribonuclease (RNase L). Although there have been many studies of the antiproliferative activity of IFN, very few have sought to relate it to the activity of this dsRNA-dependent enzyme. Recently, it was reported that mouse 3T3 cells deficient in RNase L were insensitive to the anti-

cellular and antiviral effects of IFN. Varying degrees of sensitivity of human lymphoblastoid cells to IFNα, β and γ appeared to correlate to some degree with the induction of 2',5'-oligo(A)synthetase.

In the initial studies in this Section with human IFN's, purified IFNβ and recombinant IFNαA were tested against human leukemic cells and colon carcinoma cell line HT-29. These studies indicated that all cell lines with the exception of HT-29 were resistant to the growth inhibitory effects of IFN. In HT-29 cells, IFNβ and IFNαA were cytostatic but not cytocidal. IFNβ and IFNαA were potentiated by coadministration of the synthetic dsRNA, $I_n \cdot C_n$, and this regimen not only doubled growth inhibition but also resulted in a 30-40% reduction in colony formation. The 2',5'-oligo(A) pathway was assessed by measuring 2',5'-oligo(A) synthetase and 2',5'-oligo(A) phosphodiesterase activities. While the latter activity was unaffected by IFN or $I_n \cdot C_n$, the synthetase was induced more than 15-fold by one or three day treatment with IFNβ or IFNαA. Significantly, phosphodiesterase activity was reduced by combined treatment with IFN + $I_n \cdot C_n$ and synthetase activity was not induced further. These results suggest that HT-29 cells were potentially capable of enhanced 2'5'-oligo(A) synthesis which may have been responsible for increased cytotoxicity resulting from the combined regimen of IFN + $I_n \cdot C_n$.

Further investigations with purified IFNγ have revealed addition clues to the significance of 2',5'-oligo(A) synthesis and cytotoxicity. HT-29 cells were extremely sensitive to IFNγ with a two log reduction in colony formation occurring after 3 days of treatment. 2',5'-Oligo(A) synthetase but not 2',5'-oligo(A) phosphodiesterase was induced 20-fold. In contrast, the human T-cell leukemia, MOLT-4, which was totally insensitive to IFNγ did not show any induction of synthetase activity. These data as a whole suggest that the IFN-dependent formation of 2',5'-oligo(A) plays a role in the antiproliferative action of IFN. The relative degree of dependence on this pathway among the various IFN's is not known at present.

The interferon-mediated induction of 2',5'-oligo(A) has produced several interesting biochemical leads which may be directly applicable to cancer and virus chemotherapy. Synthetic analogs of 2',5'-oligo(A) now comprise a new class of chemotherapeutic agents. Studies by other laboratories indicate the feasibility of this pharmacological approach, and studies of synthetic 2',5'-oligo(A) analogs will be conducted conjointly between this Section and the Drug Design and Chemistry Section.

Another important lead in the IFN area deals with the observation that mouse IFN induces a polyamine-dependent protein kinase in Ehrlich ascites cells which phosphorylates a 68,000 dalton protein. We have recently confirmed this observation with IFNγ-stimulated HT-29 cells and have further established that the protein kinase is not dsRNA-dependent. The interesting observation that phosphorylation of the 68,000 dalton enzyme, ornithine decarboxylase by a polyamine-dependent protein kinase results in the inactivation of the latter enzyme suggests that IFN may mediate the inhibition of polyamine synthesis via feedback control by the end products of this biosynthetic pathway. The relevance of this effect to the antiproliferative activity of IFN bears further examination.

Publications

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2. Earle, M.F. and Glazer, R.I.: Activity and metabolism of 2-β-D-ribofuranosylthiazole-4-carboxamide in human lymphoid tumor cells in culture. Cancer Res. 43: 133-137, 1983.

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4. Jayaram, H.N., Dion, R.L., Glazer, R.I., Johns, D.G., Robins, R.I., and Cooney, D.A.: Initial studies on the mechanism of action of a new oncolytic thiazole nucleoside 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC 286193). Biochem. Pharmacol. 31: 2371-2380, 1982.

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6. Jayaram, H.N., Smith, A.L., Glazer, R.I., Johns, D.G., and Cooney, D.A.: Studies on the mechanism of action of 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC-286193). II. Relationship between dose level and biochemical effects of P388 leukemia in vivo. Biochem. Pharmacol. 31: 3839-3845, 1982.

7. Johnston, J.B. and Glazer, R.I.: Pharmacologic studies of 3'-(4-morpholinyl)-3'-deaminodaunorubicin in human colon carcinoma cells in vitro. Cancer Res. 43: 1044-1048, 1983.

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9. Glazer, R.I. and Hartman, K.D.: In vitro translation of messenger RNA following exposure of human colon carcinoma cells in culture to 5-fluorouracil and 5fluorouridine. Mol. Pharmacol. 23: 540-546, 1983.

10. Chapekar, M.S. and Glazer, R.I.: The effects of fibroblast and recombinant leukocyte interferons on (2',5')oligo(A) synthesis and cell proliferation in human colon carcinoma cells in vitro. Cancer Res. 43: in press, 1983.

11. Johnston, J.B., Zwelling, L., Kerrigan, D., Lloyd, L.S., and Glazer, R.I.: Comparison of DNA scission and cytotoxicity produced by Adriamycin and 5-iminodaunorubicin in human colon carcinoma cells. Biochem. Pharmacol. 31: in press, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical & Pharmacologic Studies with Oncolytic Nucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

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David A. Cooney, Head, Biochemistry Section, LMCB, DTP, DCT, NCI

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Biochemistry Section has continued to examine the biochemical and pharmacologic factors governing the responsiveness of neoplastic cells to the oncolytic nucleosides: tiazofurin, selenazofurin and arabinosyl-5-azacytidine (araAC) I. Factors governing the responsiveness of tumors to tiazofurin have been studied in the six transplantable lines which formerly constituted the NCI Rodent Tumor Panel, as well as in six cultured human lung cancers at the NCI-Navy. In both series, tumors or cells responsive to tiazofurin accumulated markedly more of the active metabolite TAD (tiazofurin adenine dinucleotide) than their resistant counterparts. Sensitive cells also showed significantly greater depressions in their guanosine nucleotide pools as a consequence of more profound inhibition of IMP dehydrogenase. These experiments warrant the anticipation that measurements of the extent of anabolism of tiazofurin to TAD ought to provide information of prognostic value in clinical trials with this agent. II. Selenazofurin is a new analog of tiazofurin wherein selenium replaces sulfur. Studies have established that the drug is 5 times more potent than tiazofurin in killing cultured P388 cells, and is anabolized to a dinucleotide (SAD) more extensively than tiazofurin. This dinucleotide has now been synthesized chemically and characterized by NMR and mass spectrometry. Kinetically, it is approximately 5 times more potent than TAD with a inhibitory constant of 50 nM. III. AraAC is extensively phosphorylated in native P388 cells, but not at all in a variant, resistant line developed in our laboratory. This resistance has been traced to a deletion of deoxycytidine kinase. The resistant line is cross-resistant to araC and deoxy-5-AC, but not to 5-AC. AraAC is deaminated by renal cytidine deaminase but this enzyme is apparently absent from both the native and variant lines. After anabolism, the drug is also incorporated into nucleic acids: however, in vivo, using tumors of the Rodent Tumor Panel, the extent of such incorporation did not correlate with sensitivity or resistance to araAC.

I. Studies with Tiazofurin

In last year's annual report, one mechanism of action of the novel 'C' nucleoside, tiazofurin was described: in susceptible cells (P388 leukemia), the drug was anabolized to an analog of NAD in which the nicotinamide moiety is replaced by the thiazole-4-carboxamide ring. This fraudulent coenzyme (abbreviated TAD) strongly inhibits IMP dehydrogenase thereby restricting the quantity of guanosine nucleotides and deoxyribonucleotides available for nucleic acid biosynthesis. Also described was the development of a variant of the P388 leukemia exhibiting strong and stable resistance to tiazofurin in vitro and in vivo; this variant, designated P388/TR, fails to anabolize tizofurin to TAD - a failure attributable, in turn, to a deficiency of NAD pyrophosphorylase.

During the past year, additional studies have been undertaken to determine whether the mechanism of resistance elucidated in the P388/TR cells under artificial circumstances (i.e. in the face of a tremendous selection pressure) were of general occurrence. To examine this point, the metabolism of the drug has been studied in the six neoplasms which had constituted the NCI transplantable rodent tumor panel, 3 of these being sensitive, 3 being resistant to tiazofurin treatment. The results of these studies are condensed in Table 1, where it can be seen that there is a distinct positive correlation between the extent of formation of TAD, the disturbance in guanosine nucleotide biosynthesis and the native responsiveness of the rodent tumors to tiazofurin.

Table 1

Tumor	Sensitivity to TR	TAD (nmoles/g)	NAD-Pyrophosphorylase/ TADase (nmoles/mg/hr)	Adenosine nucleotides (% of Control)	Guanosine nucleotides (% of Control)	IMP (% of Control)
P388/S	S	3.9 ± 0.6	3.31	100	49	737
Lewis lung	S	19.0 ± 3.9	1.90	69	45	413
L1210	S	11.7 ± 2.0	3.60	69	39	365
B16	R	1.3 ± 0.2	0.47	91	57	766
Colon 38	R	0.9 ± 0.1	0.26	84	69	94
M5076	R	0.6 ± 0.1	0.25	75	68	188
P388/TR	R	<0.05	0.46	100	100	100

Persuant to these in vivo studies, attempts were made to determine the factors that govern the responsiveness of cultured human tumors to tiazofurin. The results of these studies, summarized in Table 2, warrant the conclusion that here too, the extent of anabolism of tiazofurin to TAD correlates positively with the sensitivity of human lung tumor cells to the drug.

Although the correlations described in Tables 1 and 2 are encouraging, they are not perfect. In the forthcoming year, attempts will be made to determine which factors other than accumulation of TAD might be responsible for moderating the therapeutic activity of tiazofurin.

Table 2

Cell line (Histology)	Colony survival[a] (%)	TAD accumulation[a] (pmoles/10^6 cells)	IMP dehydrogenase inhibition (%)	IMP (% of Control)	Guanosine Nucleotides (% of Control)
NCI H82 (Large cell)	0	1110	98	7500	43
NCI N417 (Large cell)	1.5	1510	96	1017	25
NCI H23 (AdenoCA)	0	2100	73	1375	29
NCI H249 (Small cell)	50	370	74	1000	29
NCI H146 (Small cell)	75	47	17	424	86
NCI H125 (AdenoCA)	75	263	59	382	82

[a] TR concentration: 0.1 mM

II. Studies with Selenazofurin

Selenazofurin is a novel "C" nucleoside patterned after tiazofurin except that selenium replaces sulfur in the heterocyclic ring. Versus P388 cells in culture, selenazofurin is ~5 times more cytotoxic than its prototype; the drug is also anabolized to a dinucleotide, selenazofurin adenine dinucleotide, (SAD) ~5 times more efficiently than tiazofurin. However, P388 cells resistant to tiazofurin are fully cross-resistant to the selenium compound. The chemical synthesis and characterization of SAD are described elsewhere in this report. Using this preparation, a kinetic analysis was carried out with a partially purified preparation of IMPD from P388 cells: using NAD as the variable substrate, SAD exerted non-competitive exhibition, with a K_i of 5×10^{-8}M; this value is ~5 times lower than that of TAD.

III. Studies with Arabinosyl-5-Azacytidine (AraAC)

AraAC, a novel nucleoside synthesized in LMCB, incorporates the structural elements of two extensively studied antimetabolites effective in the control of human cancer: cytarabine (Ara-C) and 5-azacytidine (5-AC). Studies with this agent have been initiated, using P388 cells in culture, and tumors of the NCI panel in vivo. In vitro, exposure to the drug arrests cells in the S-phase of the cell-cycle and selectively inhibits DNA synthesis. Deoxycytidine totally prevents these effects. Using tritiated drug, it has been demonstrated that no deamination of Ara C transpires, and that THU fails to potentiate its cytotoxicity versus P388 lymphoblasts.

Vigorous phosphorylation to mono- and di- and triphosphates is, however, carried out by these cells, and the drug is rather extensively incorporated into nucleic acids.

A variant of the P388 leukemia (P388/AraAC) solidly resistant to AraAC was developed in vitro by exposure to incremental concentrations of drug over 20 generations. This variant fails to phosphorylate both AraAC and deoxycytidine and so is concluded to be deficient in deoxycytidine kinase. Accordingly, it is cross-resistant to AraC, and deoxy-5-AC but not to 5-AC.

In vivo pulsing of transplantable tumors and xenografts with AraAC results in extensive phosphorylation and incorporation into nucleic acids in all cases, with no obvious correlation to the sensitive or resistant nature of the tumors.

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2. Jayaram, H.N., Dion, R.L., Glazer, R.I., Johns, D.G., Robbins, R.K., Srivastava, P.C., and Cooney, D.A.: Initial studies on the mechanism of action of a new oncolytic thiazole nucleoside, 2-β-D-ribofuranosylthiazol-4-carboxamide (NSC 286193). Biochem. Pharmacol. 31: 2371-2380, 1982.

3. Jayaram, H.N., Smith, A.L., Glazer, R.I., Johns, D.G., and Cooney, D.A.: Studies on the mechanism of action of 2-β-D-ribofuranosylthiazole-4-carboxamide (NSC 296193). II. Relationship between dose-level and biochemical effects in P388 leukemia, in vivo. Biochem. Pharmacol. 31: 3839-3845, 1982.

4. Cooney, D.A., Jayaram, H.N., Gebeyehu, G., Betts, C.R., Kelley, J.A., Marquez, V.E., and Johns, D.G.: The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase inhibitory properties. Biochem. Pharmacol. 31: 2133-2136, 1982.

5. Jayaram, H.N., Cooney, D.A., Glazer, R.I., Dion, R.L., and Johns, D.G.: Mechanism of resistance to the oncolytic C-nucleoside 2-β-D-ribofuranosylthiazole-4carboxamide (NSC-28193). Biochem. Pharmacol. 31: 2557-2560, 1982.

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7. Ardalan, B., Jamin, D., Gala, K., Presant, C. and Jayaram, H.N.: Phase I clinical and biochemical study of acivicin (AT-125). Cancer, in press, 1983.

8. Cooney, D.A., Jayaram, H.N., Glazer, R.I., Kelley, J.A., Marquez, V.E., Gebeyehu, G., Van Cott, A.C., Zwelling, L.A., Johns, D.G.: Studies on the mechanism of action of tiazofurin. Metabolism to an analog of NAD with potent IMP dehydrogenase-inhibitory activity. In Weber, G. (Ed.): Advances in Enzyme Regulation, New York, Pergamon Press, in press, 1983.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM06153-01 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Pharmacology of Antineoplastic Agents and Other Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

N.R. Bachur, Medical Research Officer, Lab. of Med. Chem. & Biology, NCI

COOPERATING UNITS *(if any)*

University of Maryland Cancer Center

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The plasma and urinary pharmacokinetics of adriamycin were examined in 6 sarcoma patients. Drug was administered by prolonged continuous infusion. Peak adriamycin concentrations are reduced by this infusion but total drug exposure is not compromised. The metabolism and disappearance of marcellomycin was studied in 6 patients with normal hepatic and renal function. No correlation among pharmacokinetic parameters and patient toxicities was found. Important differences in metabolism and elimination between marcellomycin and aclacinomycin A were noted.

A. Prolonged continuous infusion (Cl) of ADR has been shown to diminish microscopic changes of myocardial damage and possibly to reduce incidence or to delay onset of clinical congestive heart failure. Lowering of peak plasma Adr concentrations is one postulated mechanism for this protection. We examined plasma and urinary pharmacokinetics of Adr (45-75 mg/M^2) in 6 sarcoma patients during 10 courses of Adr given by 96 hr Cl. We utilized specific fluorescence and thin layer chromatography to assay Adr and metabolites. Mean exposure (±S.D.) (AUC) to Adr plus metabolites was 15.77 ± 3.32 μM-hr, a value consistent with AUC's folowing bolus Adr injection. The majority of exposure occurred during infusion (11.73 μM-hr). AUC for Adr was 5.8 ± 1.63 μM-hr (4.52 during infusion vs. 1.3 during first 24 hr post-infusion), and that for adriamycinol (Aol) was 2.76 ± 0.74 μM-hr (2.2 vs. 0.56). Peak concentration for Adr plus metabolites, Adr, and Aol were, respectively, 0.246 ± 0.073, 0.092 ± 0.035, and 0,044 ± 0.022 μM. These values are 10% or less of peak concentrations expected following bolus Adr. Urinary excretion of Adr plus metabolites in 5 days varied from 10%-40% of dose, values much higheer than those for bolus Adr. Adr was the predominant species during infusion (>75% of total anthracycline) but metabolites accounted for >50% of recovered urinary fluorescence post infusion. Our data demonstrate that peak plasma Adr concentrations are reduced during Cl, but total plasma Adr exposure is not compromised. The majority of exposure to Adr and metabolites occurs during Cl. Increased urinary excretion of Adr and metabolites may reflect changes in relative contributions of hepatic vs. urinary elimination during Cl, the effects of prolonged maintenance of plasma Adr concentrations above some critical threshold during Cl, or an altered volume of distribution for Adr.

B. We have investigated the metabolism and disappearance of marcellomycin M, a novel Class II anthracycline in 6 patients with normal hepatic and renal function, and no thirdspace fluid accumulations. M (40-5- mg/m^2) was infused i.v. over 15 min. Plasma and urine samples were collected for up to 72 hrs. M and metabolites were separated by thin layer chromatoggraphy and quantified by specific fluorescence. Disappearance of total M-derived fluorescence (TF) from plasma followed first order kinetics and lacked the rebound in TF previously reported for the structurally similar agent, acacinomycin A. Peak plasma M concentration (mean ± S.D.) was 1.67 ± 0.61 μM. M disappeared in bi- or tri-exponential fashion, and plasma concentration-time plot (AUC), including infusion time, was 0.866 ± 0.212 μM-hr. Plasma clearance of M was 1.92 ± 0.57 L/min. Three metabolites of M were consistently observed a polar conjugate (P1) and 2 aglycones (G1 and G2). The peak concentrations of the metabolites were 25% or less than that of M, but their persistence in plasma resulted in higher AUC's compared to M. Urinary excretion of TF amounted to 7.7 ± 1.6% of dose. The predominant urinary species was M (73%). P1 and the aglycones represented, respectively, 9.3% and 3% of excreted anthracyclines. 94% of TF appeared in urine by 12 hrs after infusion. We could discern no correlation among pharmacokinetic parameters and the various toxicities encountered in these patients. We conclude that there are important differences in metabolism and elimination beteen M and aclacinomycin A. Our study does not provide a ready pharmacological basis for the erratic toxicities of M noted in Phase 1 clinical trials.

This is a continuing project from the Baltimore Cancer Research Program.

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1. Konits, P.H., Egorin, M.J., Van Echo, D.A., Aisner, J., Andrews, P.A., May, M.E., Bachur, N.R., and Wiernik, P.H.: Phase II evaluation and plasma pharmacokinetics of high-dose intravenous 6-thioguanine in patients with colorectal carcinoma. Cancer Chemother. Pharmacol. 8: 199-203, 1982.

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3. Andrews, P., Egorin, M., May, M., and Bachur, N.R.: 6-Thioguanine pharmacology in human: Areversed-phase HPLC approach. J. Chromat. Biomed. Applic. 227: 83-91, 1982.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

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TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biochemical Pharmacology of Anti Cancer and Other Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

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University of Maryland Cancer Center

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD, 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Specific anthracycline binding proteins were identified in mouse liver by photoaffinity labeling with a photoactivatable daunorubicin analogue. The influence of these cytoplasmic receptors on subcellular disposition, metabolism and antineoplastic activity was studied. The metabolism and disposition of marcellomycin in mice was examined. The presence of a previously unrecognized enzyme accounting for a major biotransformation to non-fluorescent metabolite(s) was identified.

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Other Investigators:

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Specific anthracycline antibiotic-macromolecular interactions in cells are identified by in vitro & in situ photoaffinity labeling with the radioactive photoactivatable anthracycline antibiotic analogue, N-([3H]-p-azidobenzoyl)-daunorubicin([3H]-NABD). [3H]-NABD was added to the 105,000 x g supernatant of DBA/2 mouse liver homogenates and activated with UV light. The mixture was electrophoresed on sodium dodecyl sulfate polyacrylamide gels, the gels cut into slices and the radioactivity of each slice counted. Two polypeptides of 26 and 52 kilodaltons were identified in cytoplasmic but absent in nuclear extracts. Radioactivity was preferentially incorporated into the 26 kdalt polypeptide. Anthracycline binding specificity was confirmed by competition studies utilizing 3H-NABD in the presence of various unlabeled anthracycline analogs (daunorubicin (D), N,N-dibenzyl-D, N-acetyl-D and aclacinomycin A) and the daunosamine sugar-azidobenzoyl fragment from NABD. The concentration of unlabeled competitor which decreased 3H-NABD incorporation by 50% was determined. The most competitive analog was N,N-dibenzyl-D, requiring a 200 fold excess concentration. Other anthracycline competitors required a 10^4-10^6 fold excess except for aclacinomycin A which did not compete at all. In contrast to these analogs, azidobenzoyl-daunosamine lacking the anthraquinone ring, bound preferentially to the higher molecular weight polypeptide. These studies establish the 26 kdalt polypeptide molecular binding requirements as including both the tetracyclic ring and daunosamine sugar since in the absence of the ring structure (azidobenzoyl-daunosamine) and in the presence of a class II anthracycline (aclacinomycin A), there was no competition.

We have investigated the metabolism and disposition in mice of marcellomycin (M), a new class II anthracycline undergoing clinical trial. M, dissolved in 5% DMSO/95% normal saline, was administered IV to mice (17.4 mg/kg). At specified times after injection, mice were killed, and plasma and organs were analyzed for total fluorescence (TF), M and metabolites. TF was measured after isopropanol-sulfuric acid extraction; M and metabolites were determined by TLC after chloroformisopropanol extraction. In plasma, M declined in a biexponential fashion. The estimated V_d of M was 323.4 $1/m^2$; the area under the curve (AUC) was 3.18 µgr/ml x hr and the total body clearance was 0.27 $1/min/m^2$. A polar metabolite (PI) was identified during the first 30 minutes after injection; in addition, 2 aglycones, G1 and G2, were seen. The disappearance of G1 was similar to that of M. In contrast, G2 was more persistent and represented between 35 and 40% of the TF by 8 hrs after injection. M distributed extensively to tissues. TF was initially greatest in lungs, but liver and kidney TF became equal or greater than lung TF 1 hr after injection. Brain TF was negligible. TF was intermediate in heart and muscle. By 16 hr after injection, TF in spleen was greater than that of any other organ, and remained stable to 72 hrs. M accounted for more than 75% of TF in all tissues except liver. In the liver there were significant amounts of an aglycone that had the same TLC and HPLC characteristics as 7-deoxypyrromycinone. Metabolites were cleared from organs faster than M and represented $\leq$ 10% of TF in all organs by 8 hrs after injection. Our results show that the metabolism of M in mice is qualitatively similar

to that in man. Mice have higher plasma concentrations and AUC than man for M, but much lower corresponding values for P1. The significance of these observations in explaining the discrepancies between mouse and man in M-induced myelosuppression remains to be explored.

We investigated the in vitro metabolism of M, a new class of anthracycline undergoing clinical trial. M (5 μM) was incubated at 37°C, in the presence of an NADPH generating system and an enzyme system from rat liver (whole homogenate, washed microsomes, cytosol, mitochondria, or purified NADPH cytochrome P450 reductase (P450RD)). After extraction of aliquots of reaction mixtures with chloroform-isopropanol, total fluorescence (TF) of the extract was measured, and concentrations of M and metabolites were determined by thin layer chromatography. No metabolism was seen under aerobic conditions. With anaerobic incubation, M was totally converted in 15 min to 3 aglycones (G) by rat liver homogenate, microsomes, cytosol, and mitochondria. With longer incubation times, G disappeared from the medium, with resulting loss of TF; this decrease in TF was not seen when adriamycin and daunorubicin were incubated under similar conditions. The decrease in TF was confirmed with isopropanol-sulfuric acid extraction. Boiled microsomes failed to induce the decrease of TF or G. These observations indicate that the decrease of G is not related to its binding on a subcellular component. The formation of compounds with fluorescent characteristics different from G and M was excluded by fluorescence scanning. The loss of TF did not occur with the mitochondrial preparation. P450RD preparations converted M to G, but without subsequent loss of TF. When G were the reaction substrates, the decrease of TF caused by the other enzyme systems required the presence of NADPH and anaerobic conditions. Our results suggest that G are metabolized by an oxygen-sensitive, NADPH-dependent enzyme system, different from P450RD, to non-fluorescent compounds, undetectable by usual anthracycline fluorescence assays. This metabolic pathway should be considered in interpretations of the animal and human pharmacology of M.

This is a continuing project from the Baltimore Cancer Research Program.

Publications

1. Clawson, R.E., Felsted, R.L., Bachur, N.R., and Weiner, M.: Identification of Anthracycline Binding Protein in Liver Cytosol. Fed. Proc. 1982.

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TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Control Mechanisms Affecting Cell Growth and Differentiation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

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R. L. Felsted, Research Chemist, LMCB, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Medicinal Chemistry and Biology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 10105

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell surface membrane glycoprotein structures and functions have been studied using the methods of surface labeling with I-125 or H-3 followed by two-dimensional isoelectric focusing and SDS polyacrylamide gel electrophoresis and autoradiography or fluorography. Membrane protein change during the chemical induced granulocyte differentiation of human promyelocytic leukemia HL-60 cells include the appearance of a major surface protein which is also found in normal human granulocytes and was identified as a terminal myeloid differentiation related marker. A similar analysis of a number of cytodifferentiation-inducer resistent HL-60 sublines revealed surface protein patterns in dimethylsulfoxide and 5-bromo-2'-deoxyuridine inducer resistant sublines which are very similar to wild type HL-60. In contrast retinoic acid and 6-thioguanine resistant sublines exhibited drastic differences from wild type cells. Regardless of surface pattern, upon induction of differentiation, all cell lines revealed the same newly synthesized terminal myeloid differentiation surface protein.

A. Surface membrane proteins and glycoproteins of the human leukemic cell lines K-562 and HL-60 were labeled with ^{125}I by the lactoperoxidase/H_2O_2 and 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril (Iodogen) methods. Triton X-100 extracts of the labeled cells were analyzed by isoelectric focusing and sodium dodecyl sulfate two-dimensional electrophoresis, and the labeled cell-surface macromolecules were visualized by autoradiography. The two-dimensional paterns revealed 11-12 groups of major proteins in both cell types. A comparison of apparent molecular weights and pI values indicated that at least seven labeled protein components are unique to HL-60, four are unigue to K-562, and five are common to both cell types. One of these major common labeled proteins was identified as the cell-surface membrane receptor for transferrin by absorption to transferrin-Sepharose beads. This affinity absorbed material had molecular weights of 98,000 and 200,000 under reducing and non-reducing condtions, respectively, thus confirming a dimeric structure expected for the transferrin receptor. The labeled proteins were established as cell-surface membrane components by the following criteria. 1) Identical two-dimensional patterns resulted from two labeling procedures with different surface-specific labeling mechanisms; 2) less than 4% of the individual labeled spots were coincident with the major intracellular proteins identified by Coomassie brilliant blue staining; 3) protease treatment removed up to 70% of the radioactivity from viable cells; and 4) neuraminidase treatment of viable cells resulted in a time-dependent charge shift of the labeled proteins toward more basic pH values, consistent with the removal of charged sialic acid residues from labeled surface glycoproteins. These results illustrate the value of two-dimensional electrophoresis in the study of minor and major differences of cell surface macromolecules and are consistent with the suggestion that the K-562 and HL-60 cell lines may be descendents from a common bone marrow precursor cell.

The human promyelocytic leukemia cell line HL-60 was induced to differentiate in vitro by treatment with dimethylsulfoxide or retinoic acid. Morphological maturation was accompanied by a total loss of transferrin binding and a 7-fold increase in the percentage of cells reducing nitroblue tetrazolium. Cell surface membrane proteins and glycoproteins were labeled with ^{125}I by the lactoperoxidase/ H_2O_2 or Iodogen methods and analyzed by two-dimensional isoelectric focusing and SDS-polyacrylamide gel electrophoresis and autoradiography. A minimum of twelve cell surface proteins were unchanged, three proteins (molecular weights 95,000; 87,000; and 77,000) were lost and up to seven new proteins (molecular weights 270,000; 240,000; 150,000; 135,000; 58,000; 56,000; and 50,000) appeared during HL-60 cell differentiation. The kinetics of disappearance of one major labeled cell surface protein (95,000 daltons) within two days during treatment with retinoic acid correlated with the loss of cellular transferrin binding. This protein was identified as the transferrin receptor by affinity absorption of extracts of ^{125}I surface protein-labeled cells to transferin-Sepharose beads. The affinity purified component had molecular weights of 190,000 and 95,000 daltons under non-reducing and reducing conditions, respectively, confirming its dimeric structure. Two-dimensional electrophoresis of cell surface membrane-labeled proteins of normal human granulocytes confirmed the absence of the transferrin receptor and identified cell surface proteins with molecular weight and pI values corresponding to three of the new cell surface proteins which appeared during HL-60 maturation. The most intensely labeled of these had a molecular weight of about 55,000 and was confirmed as

being identical to the corresponding 58,000 dalton HL-60 cell surface membrane protein by one-dimensional peptide mapping analysis. This prominent new 55-58,000 dalton protein increased continuously throughout retinoic acid-induced maturation and was identified as a major terminal myeloid differentiation cell surface membrane protein.

Surface membrane glycoproteins of HL-60 cell sublines which had been selected for resistance to the cytodifferentiation-inducing effects of dimethylsulfoxide (Me_2SO), retinoic acid (RA), 6-thioguanine (6TG) or 5-bromo-2'-deoxyuridine. Compared to wild-type HL-60 cells, RA- and 6TG-resistent sublines exhibited major surface glycoprotein differences including a shift in a major 150 Kilodalton HL-60 glycopetide to >200 Kilodalton. The other resistent sublines exhibited surface membrane patterns generally similar to wild type HL-60 cells. In addition, the induction of RA resistent cells with Me_2SO and Me_2SO-resistent cells with RA resulted in the appearance of the same major terminal myeloid differentiation cell surface membrane protein previously identified in wild type HL-60 cells and normal human granulocytes.

This is a continuing project from the Baltimore Cancer Research Program.

Publications

1. Felsted, R.L., Pokrywka, G., Chen, C., Egorin, M.J., and Bachur, N.R.: Radioimmunoassay and Immunochemistry of Phaseolus vulgaris phytohemagglutinin: Verification of isolectin subunit structures. Arch. Biochem. Biophys. 215: 89-99, 1982.

2. Felsted, R.L., and Gupta, S.K.: A Comparison of K-562 and HL-60 human leukemic cell surface membrane proteins by two-dimensional electrophoresis. J. Biol. Chem. 257: 13211-13217, 1982.

3. Felsted, R.L., Gupta, S.K., Glover, C.J., Fischkoff, S.A. and Gallagher, R.E.: Cell surface membrane protein changes during the differentiation of cultured human promyelocytic leukemia HL-60 cells. Cancer Res., in press, 1983.

4. Dimery, I.W., Ross, D.D., Testa, J.R., Felsted, R.L., Gupta, S.K., Pollak, A. and Bachur, N.R.: Phenotypic and cytogenetic variation of K562 cells obtained from different laboratories. Exp. Hematol., in press, 1983.

5. Gallagher, R., Felsted, R.L., Young, N., Gupta, S.K., Ferrari, H. and Testa, J.: Coordinate membrane glycoprotein, monoclonal antibody binding and cytogenetic alterations in differentiation-inducer-resistent HL-60 cells. Amer. Soc. Hematol., in press, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM06156-01 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacodynamics of Cancer Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

N. R. Bachur, Medical Research Officer, LMCB, NCI

COOPERATING UNITS *(if any)*

University of Maryland Cancer Center

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Cellular Pharmacology

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.9	0.9	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The role of one and two-electron reductions of mitomycin C on its alkylating properties was studied. One-electron reduction is sufficient to unmask drug alkylating activity while two-electron generates a different set of products. The primary mitomycin C metabolites are also reductively activated by NADPH cytochrome P450 reductase and xanthine oxidase. Whole cell reduction of AZQ to free radical anions in human K562 and HL60 and mouse L1210 cell lines was demonstrated. The penetration of AZQ and metabolytes into puppy CNS and brain tumor tissue was quantified.

A. The role of one and two-electron reduction of the quinone moiety of mitomycin C (MC) in the expression of its alkylating properties has been uncertain. To define this role we produced the radical anion and dianion of MC by electrochemical reduction in an aprotic solvent and reacted them with water under anaerobic conditions. Cyclic voltammetric (CV) analysis of MC in dimethyl formamide (DMF), 0.1 M tetraethylammonium perchlorate (TEAP) gave two cathodic waves at -0.937 and -1.410 volts (V) vs. Ag/AgCl satd. KCl, corresponding to the transfer of one and two electrons to MC. Reduction of 1.4 mM MC in DMF (0.1 M TEAP) with an electrochemical flow cell at -0.950 V yielded the radical anion as indicated by electron paramagnetic resonance (EPR) spectroscopy. EPR and CV analysis indicated that this radical was stable. Addition of this solution to water, yielded MC and 8 major products as indicated by high performance liquid chromatography (HPLC). Some of these peaks have been assigned to cochromatography with known standard as cis-and-trans-2,7-diamino-1-hydroxymitosene, 10-decarbamoyl-MC, and 2,7-diaminomitosene. Flow cell reduction of MC at -1.450 V in DMF (0.1 M TEAP) gave the dianion of MC which, when added to water, gave only two peaks by HPLC. Cochromatography with a standard and electron ionization mass spectrometry indicated that the first peak was 10-decarbamoyl-2,7-diamino-mitrosene (m/z 261 (100.0%) $M^{\pm}$, 244 (20.8%), 219 (90.9%), 203 (27.3%), 191 (30.7%)). Mass spectral analysis of the second peak indicated it was also a type of 10-decarbamoyl mitosene. These studies show that one-electreon reduction of MC is sufficient to unmask its alkylating potential and that two-electron reduction dictates the generation of a different set of products. Our data show that under the proper conditions, reductive processes causes elimination of the C10-carbamate.

B. Our earlier reports showed that mitomycin C (MC) was metabolized by NADPH cytochrome P450 reductase and xanthine oxidase. We have subsequently found that its primary metabolites, cis-and trans-2,7-diamino-1-hydroxymitosene, (cis-and trans-2d), cis-and trans-2,7-diamino-1-1phosphormitosene (cis-and trans-2d-phosphate) and 2,7-diaminomitosene are also reductively activated by both enzymes. Under identical conditions, NADPH cytochrome P450 reductase was 25 to 30 times more effective than xanthine oxidase in activating all substrates. Our observations indicate that MC can be activated beyond a primary reductive activation through primary metabolites which may be important to the mechanism of action of MC.

C. We have previously shown that AZQ is reduced to its free radical anion by rat liver microsomes and NADPH cytochrome P450 reductase. We investigate here the cellular activation of AZQ to its free radical anion because it is closer to the in vivo situation than enzymes alone. We investigated two human cell lines, K562 and HL60 and one murine cell line, L1210. In aerobic or anaerobic solutions, the radical is present immediately in all cell lines. K562 cells yielded the most radicals, HL60 produced the least, and L1210 cells produced an intermediate amount. Simultaneous oxygen consumption and EPR experiments indicate that in L1210 cells, the free radical appears while the solution contains about 50-60% oxygen. As the solution becomes anaerobic, the free radical intensity increases by approximately 85%. The kinetics of AZQ oxygen comsumption with L1210 cells yield Vmax = 2.0×10^{-8} moles of O_2 utilized/min/mg protein and $K_m = 65 \times 10^{-6}$M. Azide did not prevent free radical formation, but iodoacetate, diamide and N-ethylmaleimide prevent the formation of free radicals at equimolar concentrations.

An important finding was that boiled L1210 cells still reduce AZQ to AZQ$^{\bullet}$. Steady state quantitative measurements indicate that only 1% of the total AZQ concentration is activated to AZQ$^{\bullet}$.

D. AZQ, an antineoplastic drug designed to penetrate the blood brain barrier, has demonstrated activity against CNS neoplasms. 4-hr infusions of ^{14}C AZQ (0.8 mg/kg) were given via the left common carotid artery (IA) or left brachial vein (IV) to two groups of puppies. A third group harboring a transplantable canine glioma, received ^{14}C AZQ by IV infusion. $CHCl_3$-extractable (AZQ) and total (AZQ and metabolites) ^{14}C were determined in serial samples of plasma and CSF. At infusions' end, ^{14}C was determined in brain and tumor. I.A. infusion caused no histologic abnormalities in retina or brain. Our data show that AZQ enters CNS and brain tumor tissue in substantial concentrations and that there is no significant advantage to intracarotid infusion of AZQ. Tumor and surrounding brain contained similar concentrations of parent drug but there were higher concentrations of metabolites in tumor. This may reflect different metabolism of AZQ within brain and tumor or different permeability to metabolites.

This is a continuing project from the Baltimore Cancer Research Program.

Publications

1. Spiegel, J.F., Egorin, M.J., Collins, J.M., Lerner, B.D. and Bachur, N.R.: The murine disposition and pharmacokinetics of the antineoplastic agent, diaziquone (NSC 182986). Drug Metab. Disp. 11: 41-46, 1983.

2. Bachur, N.R.: Current Status and Future Development. In Mathe, Maral and DeJager (Eds): Mechanisms of Action of the anthracycline antibiotics. Anthracyclines. New York, Masson Publishing U.S.A., INC., 1983.

3. Bachur, N.R.: Intracellular Distribution of Antracycline Antibiotics. In Mathe, Maral, and DeJager (Eds): Mechanisms of Action of Anthracycline Antibiotics. New York, Masson Publishing U.S.A., Inc. 1983.

4. Gutierrez, P.L., Gee, M.V., and Bachur, N.R.: Kinetics of anthracycline antibiotic free radical formation and reductive glycosiidase activyt. Arch. Biochem. Biophys. (in press) 1983.

5. Gutierrez, P.L., and Bachur, N.R.: Free radicals in quinone containing antitumor agents: The nature of the diaziquone (3,6,-diaziridinyl-2,5-bis(carboethoxyamino)-1,4-benzoquinone) free radical. Biochim. Biophys. Acta (in press), 1983.

6. Gutierrez, P.L., Egorin, M.J., Fox, B.M., Friedman, R., and Bachur, N.R.: Cellular activation of diaziquone (3,6-diaziridinyl-2,5-bis(carboethoxyamino)1,4-benzoquinone) to its free radical species (submitted for publication) Br. J. Cancer, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM03580-14 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical Research in the Development of New Anticancer Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

J. S. Driscoll, Head, Drug Design and Chemistry Section, LMCB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Design and Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.2	5.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. The following topics are of current interest: (1) purine nucleosides as antitumor agents and transition-state inhibitors of purine nucleoside phosphorylase, (2) new approaches towards the synthesis of the antitumor-active antibiotic neplanocin and related analogs, (3) synthesis of pyridone nucleosides as antitumor agents and potential inhibitors of cytidine triphosphate synthetase, (4) a ring-expansion approach to novel diazepinone nucleosides, (5) synthesis of diazepinone nucleoside analogs as potential antitumor agents and inhibitors of cytidine deaminase, (6) dinucleotide analogs structurally related to NAD as inhibitory agents of inosine monophosphate dehydrogenase.

Other Investigators:

R. W. Fuller	Chemist	LMCB	NCI
G. Gebeyehu	Visiting Fellow	LMCB	NCI
M-I Lim	Cancer Expert	LMCB	NCI
D. Mao	Visiting Fellow	LMCB	NCI
V. E. Marquez	Visiting Scientist	LMCB	NCI
K. V. B. Rao	Visiting Fellow	LMCB	NCI

Project Description:

General Objective:

The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. Medicinal chemical research is directed toward the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DTP screening program, the literature, and biochemical rationale.

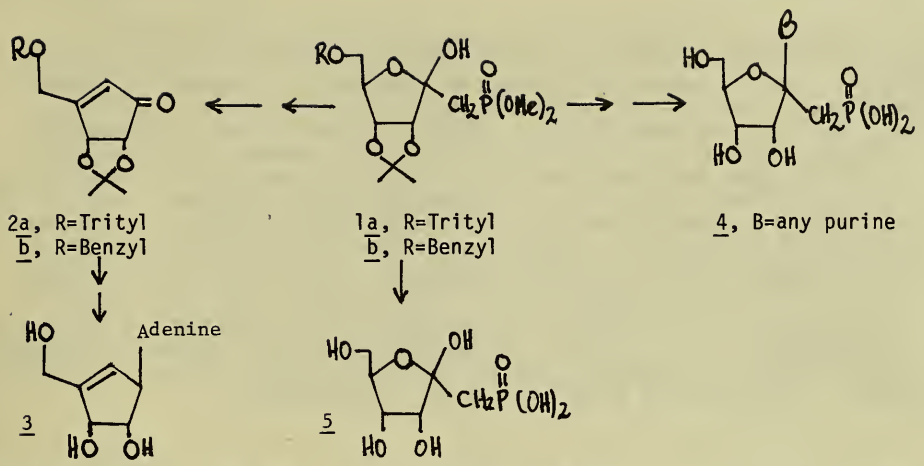
Specific Objectives:

1. Transition-state inhibitors of purine nucleoside phosphorylase (PNP) and synthesis of the antitumor antibiotic neplanocin.
2. Synthesis of 2- and 4-pyridone analogs.
3. Novel diazepinone nucleosides via ring expansion reactions.
4. Transition-state inhibitors of cytidine deaminase.
5. Dinucleotide analogs of NAD.

Major Findings:

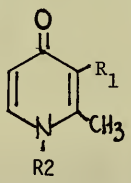
Transition-State Inhibitors of Purine Nucleoside Phosphorylase (PNP) and Synthesis of the Antitumor Antibiotic Neplanocin (Drs. Lim and Marquez):

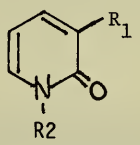
The synthetic chemistry designed for this project permitted the development of a key intermediate 1 useful for the synthesis of both neplanocin (3) and a series of purine nucleoside phosphonates capable of behaving as transition-state inhibitors of PNP (i.e. structure 4). The synthesis of the 2-cyclopenten-1-one moiety (2a,b) with the same stereochemistry as in neplanocin has been achieved



from D-ribose in nine steps. The synthesis of neplanocin is currently progressing towards its goal. Simultaneous efforts to convert 1a,b to the corresponding nucleoside derivative 4 are under development. The completely deprotected sugar phosphonate 5 will be included in the assays against PNP.

Synthesis of 2- and 4-Pyridone Analogs (Drs. Mao, Marquez and Driscoll): This project is nearing completion. In our previous report only in vitro data was reported for some of these compounds due to the limited amounts of material. After optimizing the syntheses and chromatographic separations we have been able to obtain complete in vivo data for all of the compounds prepared. With the exception of 7d, which possessed only marginal activity against murine P388 leukemia, all of these compounds proved to be inactive. The ribo and arabinofuranosyl derivatives (6a,b and 7a-c) were designed as 3-deazauridine analogs and hence as potential inhibitors of cytidine triphosphate synthetase.

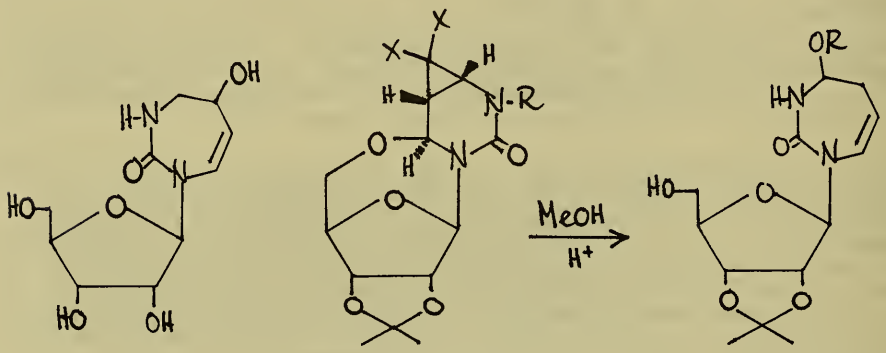




6a, R_1=OH, R_2=β-D-ribofuranosyl
b, R_1=OH, R_2=β-D-arabinofuranosyl

7a, R_1=OH, R_2=β-D-ribofuranosyl
b, R_1=OMe, R_2=β-D-ribofuranosyl
c, R_1=OH, R_2=β-D-arabinofuranosyl
d, R_1=OH, R_2=CH_2CH_2OH
e, R_1=OH, R_2=CH_2CH_2Cl
f, R_1=OMe, R_2=CH_2CH_2Cl
g, R_1=OAc, R_2=CH_2CH_2Cl
h, R_1=OH, R_2=$CH_2CH_2NH_2$
i, R_1=OBn, R_2=CH_2CH_2SH

Novel Diazepinone Nucleosides via Ring Expansion Reactions (Drs. Rao and Marquez): This project which was started two years ago has now been successfully completed. The ring expansion approach has permitted the synthesis of unknown 4-hydroxy-1,3-diazepin-2-one nucleoside (10b) which is isomeric to the very powerful cytidine deaminase inhibitor 8 (5-hydroxy analog) previously made by us. This compound has also proven to be a pivotal intermediate for the generation of a series of new diazepinone nucleosides. In regard to the chemistry, this approach represents the first ring expansion reported for this ring system to occur at room temperature and with quantitative efficiency. The key intermediate 9b, obtained as a single product after two reactions, constitutes also a very unique case in which three asymmetric centers were introduced in a molecule with complete stereoselectivity. The structure of 9b was resolved by X-ray analysis. After removal of the halogens and the N-benzoyl group in 9c, the resulting bicyclic 9e produced the diazepinone 10a in methanol under mild acid catalysis. Exchange of the MeO group by OH was performed in refluxing water. The biological activity is under investigation.

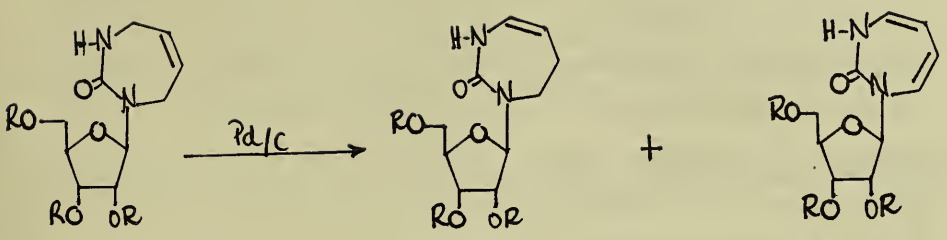


8

9a, R=OCØ, X=Cl
b, R=H, X=Cl
c, R=OCØ, X=Br
d, R=OCØ, X=H
e, R=H, X=H

10a, R=Me
b, R=H

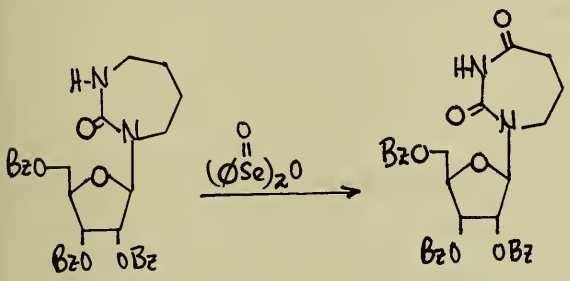
Transition-State Inhibitors of Cytidine Deaminase (Dr. Marquez): We have continued to explore the chemistry of the new ring system of 1,3-diazepin-2-one nucleosides with the idea of delineating structure-activity relationships and with the hope of finding new leads in antitumor activity. Two new reactions discovered in our laboratory have given access to a series of isomeric diazepinones which have been tested as cytidine deaminase (CDA) inhibitors.



11a, R=Bz
b, R=H

12a, R=Bz
b, R=H

13a, R=Bz
b, R=H



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15

Compounds 12b and 13b behaved as potent CDA inhibitors (K_i $8x10^{-8}$ and $2x10^{-7}$M, respectively) of the human liver enzyme. The additional advantage over the previously made analogs (i.e. 11b) is that both compounds 12b and 13b possess strong UV activity with no loss in biological potency. This property will facilitate the study of the drugs' metabolism. the second reaction involving the preparation of the 4-keto compound 15 is under study.

Dinucleotide Analogs of NAD (Drs. Gebeyehu and Marquez): Following our successful synthesis of the tiazofurin-containing NAD analog (16, TAD), other derivatives have been synthesized. The purpose of this project is to design more powerful and stable coenzyme analogs which behave as inhibitors of inosine monophosphate dehydrogenase (IMPD). Of the three different approaches that have been followed only the compounds that have been made and fully characterized will be listed.

	IMPD-inhibition	
	K_i	ID_{50}
1. Changes in the heterocyclic base		
16, tiazofurin-P-P-adenosine	0.12μM	-
17, selenazofurin-P-P-adenosine	0.055μM	-
18, virazole-P-P-adenosine	-	-
2. Changes in the adenosine moiety		
19, tiazofurin-P-P-2′-d-adenosine	-	-
3. Changes in the phosphodiester linkage		
20, tiazofurin-CH_2-P-P-adenosine	-	0.53μM
21, tiazofurin-P-P-CH_2-adenosine	-	-
4. Miscellaneous		
22, tiazofurin-CH_2-P-P-CH_2-tiazofurin	-	26μM

The complete biological study is in progress and several other new analogs are being currently prepared.

Publications:

1. Cooney, D.A., Jayaram, H.N., Gebeyehu, G., Betts, C.R., Kelley, J.A., Marquez, V.E. and Johns, D.G.: The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase-inhibitory properties. Biochem. Pharmacol. 31, 2133-2136, 1982.

2. Marquez, V.E.: Antineoplastic agents. Hess, H.J. (Eds.) In: Annual Reports in Medicinal Chemistry, Vol. 17, Academic Press, New York, 1982, pp. 163-174.

3. Rao, K.V.B., Marquez, V.E., Kelley, J.A. and Corcoran, M.T.: A new synthesis of 3-(β-D-ribofuranosyl)uracil (Isouridine) via the intermediary of an O^6,5'-cyclotetrahydropyrimidinone nucleoside. J. Chem. Soc. [Perkin I], 127-130, 1983.

4. Vistica, D.T., Fuller, R., Dillon, N., Petro, B.J.: Comparative reactivity of cyclic amino acids with system L in murine L1210 leukemia cells and murine bone marrow progenitor cells (CFU-C): A potential basis for selective drug design. Rational Basis for Chemotherapy, pp. 475-485. Alan R. Liss, Inc., New York, New York, 1983.

5. Marquez, V.E.: Mechanisms of formation of cyclic urea nucleosides. 2. Direct N-glycosylation versus O- to N-transglycosylation. Nucleos. Nucleot. (in press).

6. Gebeyehu, G., Marquez, V.E., Kelley, J.A., Cooney, D.A., Jayaram, H.N. and Johns, D.G.: Synthesis of thiazole-4-carboxamide-adenine dinucleotide (TAD). A powerful inhibitor of IMP-dehydrogenase. J. Med. Chem. (in press).

7. Cooney, D.A., Jayaram, H.N., Glazer, R.I., Kelley, J.A., Marquez, V.E., Gebeyehu, G., Van Cott, A., Zwelling, L.A., Johns, D.G.: Studies on the mechanism of action of tiazofurin. Metabolism to an analog of NAD with potent IMP dehydrogenase-inhibitory activity. Adv. Enz. Reg. (in press).

8. Marquez, V.E.: Antineoplastic Agents. Hess, H.J. (Eds.) In: Annual Reports in Medicinal Chemistry, Vol. 18, Academic Press, New York, 1983, (in press).

Patents:

1. Marquez, V.E., Cooney, D.A., Gebeyehu, G., and Jayaram, H.N.: Inosine-5'-monophosphate (IMP) inhibitors. Submitted to U.S. Patent Office Sept. 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM03581-14 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Analytical Chemistry of New Anticancer Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

J. A. Kelley, Research Chemist, LMCB, NCI

COOPERATING UNITS *(if any)*

Medicine Branch and Clinical Pharmacology Branch, COP, DCT, NCI

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Design and Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.6	2.0	.6

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the research and development of analytical methods which are used to: (1) establish the structure and purity of new antitumor agents and their metabolites, (2) determine physical and chemical properties of new anticancer drugs, (3) quantitate drugs and their metabolites in biological samples to elucidate pharmacology and to determine pharmaco-kinetics, and (4) study reaction mechanisms of potentially useful synthetic transformations. Mass spectrometry, gas chromatography and high-performance liquid chromatography, either alone or in combination, are emphasized techniques. Compounds of current interest are aziridinylbenzoquinones, reduced pyrimidine nucleosides, mono- and dinucleotides, nitrogen mustards, and cytidine deaminase inhibitors. The acid-catalyzed rearrangements of reduced pyrimidine and diazepinone nucleosides continue under investigation.

Other Investigators:

J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI
P. N. Huguenin	Visiting Fellow	LMCB	NCI
J. S. Roth	Chemist	LMCB	NCI

Project Description:

General Objective:

The objective of this project is the research and development of analytical techniques for establishing the structure and purity of new anticancer drug candidates, determining their important physical and chemical properties, elucidating structures of metabolites of new antitumor agents, measuring these drugs and their metabolites in physiological samples and studying reaction mechanisms. Mass spectrometry (MS), gas chromatography (GC), high-performance liquid chromatogrpahy (HPLC) and the combination of these techniques (GC/MS, LC/MS) are the emphasized methods. Other analytical methods such as NMR, UV and IR spectroscopy are also employed.

Specific Objectives:

1. Human pharmacokinetics of AZQ (Diaziquone) in a Phase II clinical trial.
2. Analysis of 5,6-dihydro-5-azacytidine (DHAC) and determination of human pharmacokinetics.
3. Analytical methods development for spirohydantoin mustard (SHM).
4. Development of a rapid assay for hexamethylene bisacetamide (HMBA) and application to preclinical pharmacology studies.
5. Synthetic and collaborative project support.

Major Findings:

Human Pharmacokinetics of AZQ (Diaziquone) in a Phase II Clinical Trial (Drs. Kelley, Curt and Collins, MB & CPB-COP):

Thirty patients with high grade gliomas, who had documented evidence of tumor regrowth, were treated with 20 mg/m^2 of AZQ given iv on days 1 and 8 of a 28 day cycle. A previously developed HPLC assay was employed to determine both intra- and post-infusion plasma AZQ levels in 5 patients given the drug as a constant rate iv infusion of limited duration. Plasma drug levels were also measured in 2 patients for whom there were brain tumor biopsy specimens, and AZQ plasma protein binding was investigated in an additional 2 patients as a function of drug concentration. Characterization of the concentration versus time profile during the infusion period gave a more accurate estimate of area under the curve and showed that the majority of plasma drug exposure occurs during this time when the infusion is longer than 30 min. A more precise value of 467 ± 57 ml/min/m^2 was obtained for total body clearance, reflecting the

fact that area under the curve was known with greater certainty. Although technical problems were encountered in determining plasma protein binding by membrane ultrafiltration because of variable membrane effects, a consistently high binding (75-99%) was noted for AZQ in actual patient samples. This strong plasma binding which restricts distribution may account for a volume of distribution corresponding to only 28% of body space. The first evidence that unchanged AZQ penetrates neoplastic brain tissue in addition to crossing the blood-brain barrier was seen in tumor cyst fluid levels that approximated the corresponding plasma levels and in a substantial drug level in the peripheral tumor of one patient.

Analysis of 5,6-Dihydro-5-azacytidine (DHAC) and Determination of Human Pharmacokinetics (Drs. Huguenin, Kelley, Curt and Collins, MB & CPB-COP):

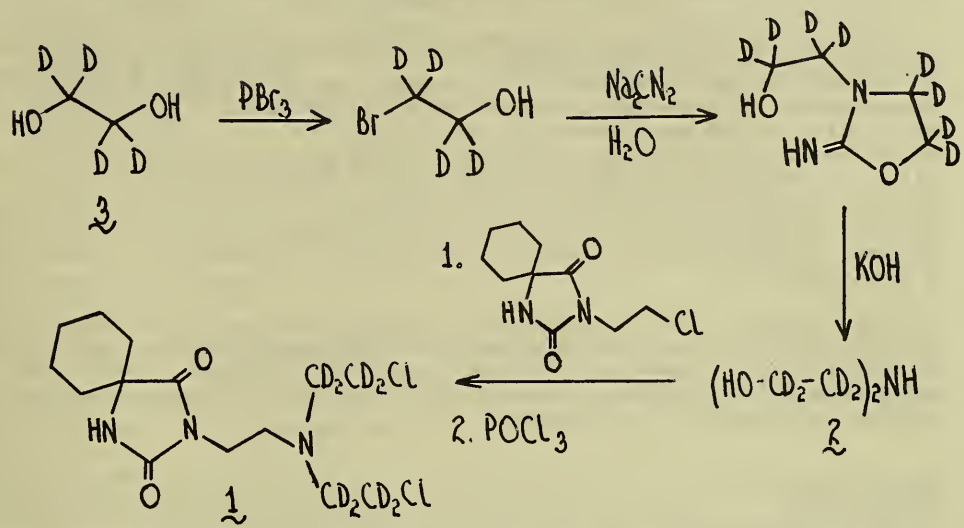
A sensitive and selective reverse phase HPLC assay has been developed for DHAC to allow measurement of concentrations as low as 50 ng/ml ($2x10^{-7}M$) in plasma and urine. After addition of an internal standard, sample cleanup is carried out by ultrafiltration followed by sequential boronate gel affinity and cation exchange chromatography. DHAC is then reacted with N,N-dimethylformamide diethylacetal to form a dimethylaminomethylene derivative which is more easily detected by UV (λ_{max}=264 nm, log ε =4.3) and is better retained on a reverse phase column. This assay has been validated in a rat model where the animals were given a single iv bolus dose of 50 mg/kg. The plasma disappearance curve of DHAC is described by a two-compartment open model where $t_{1/2}(\alpha)$=4.2 min and $t_{1/2}(\beta)$=61 min.

DHAC is also undergoing a Phase I clinical trial in which the drug is administered as a 24 hr continuous infusion once every 28 days. The drug has been found to be unstable in human plasma because of cytidine deaminase activity ($t_{1/2}$=4.0 hr at 37^{o}), thus requiring addition of an appropriate inhibitor to preserve the sample. Otherwise, analysis of patient samples has demonstrated that this assay is also well-suited for human pharmacokinetic analysis. For patients treated with 5000 mg/m^2 DHAC, steady-state plasma concentrations of 9-14 µg/ml are achieved by 8 hr. Post-infusion plasma elimination of DHAC is approximated by a two-compartment open model where $t_{1/2}$ for the terminal phase is 71±8 min. Substantial quantities of unchanged drug could be detected in urine and additional more comprehensive studies are planned to characterize its urinary excretion.

Analytical Methods Development for Spirohydantoin Mustard (SHM) (Drs. Huguenin and Kelley):

Spirohydantoin mustard (NSC 172112, Spiromustine), a new candidate antitumor agent with potential activity against central nervous system (CNS) tumors, is scheduled to undergo clinical trial in the near future. A sensitive and reliable selected ion monitoring GC/MS assay for SHM is under development in order to define both plasma elimination and penentration into the CNS. Synthesis of SHM-d_8(1) as an internal standard has been initiated in order to develop an assay with maximum sensitivity and minimum potential interferences. This has required defining a new route to diethanolamine-d_8(2), a key syn-

thetic intermediate, from readily available ethylene glycol-d_4(3).



Once the internal standard is in hand, procedures for isolation of SHM from plasma and cerebrospinal fluid will be optimized and the overall analytical procedure will be validated in an animal model.

Development of a Rapid Assay for Hexamethylene Bisacetamide (HMBA) and Application to Preclinical Pharmacology Studies (Drs. Kelley, Litterst and Ms. Roth):

HMBA induces differentiation of virtually the entire population (>99%) of murine erythroleukemia cells if concentrations of 5mM are maintained for more than 48 hr. Studies have been undertaken in rats to determine the feasibility of maintaining HMBA concentrations in vivo which match exposure conditions in vitro. A rapid and simple gas chromatographic method incorporating an internal standard and involving the direct analysis of plasma or urine has been developed for the measurement of HMBA in these fluids. A limit of detection of less than 50 µg/ml is obtained and the small amount of plasma required for sample workup permits multiple sampling from treated animals. A single iv bolus dose of 800 mg/kg HMBA results in plasma levels well above 5 mM, but the compound is rapidly eliminated from the plasma ($t_{1/2}(\alpha)$=5.5min;$t_{1/2}(\beta)$=122 min) with a total body clearance of 7 ml/min/kg. The primary route of excretion appears to be urinary since more than 65% of the administered dose is found as unchanged HMBA in the urine after the first 8 hr. Future work will be directed towards determining what HMBA plasma levels can be maintained through continuous infusion of non-toxic doses in unanesthetized animals.

Synthetic and Collaborative Project Support (Dr. Kelley):

A. Applications of Fast Atom Bombardment (FAB) Mass Spectrometry to Nucleoside and Nucleotide Structure Determination: The DDCS has an extensive program in the synthesis of new nucleosides and nucleotides (see Project Z01 CM 03580-13, Chemical Research in the Development of New Anticancer Drugs). Both positive and negative ion FAB mass spectrometry has been applied as a rapid and simple method for the analysis of these compounds without the requirement of derivatization or chemical pretreatment. The mass spectral fragmentation patterns of mono- and dinucleotides have been defined and applied to synthetic mixture analysis. Accurate mass determination of underivatized nucleosides by FAB has also been employed to obtain elemental compositions of unstable or difficult to purity compounds.

B. Miscellaneous: Numerous samples which cannot be categorized as coming from any one project area were also analyzed by the appropriate mass spectral and chromatographic techniques on an individual basis. Included in this group were anthracycline antibiotics, diazepinones, 4-pyridone derivatives, peptides, N-oxides and mitomycin C and its metabolites.

Publications:

1. Bachur, N.R., Collins, J.M., Kelley, J.A., Van Echo, D.A., Kaplan, R.S. and Whitacre, M.: Diaziquone, 2,5-diaziridinyl-3,6-biscarboethoxyamino-1,4-benzoquinone, plasma and cerebrospinal fluid kinetics. Clin. Pharmacol. Ther. 31: 650-655, 1982.

2. Flora, K.P., Cradock, J.C. and Kelley, J.A.: The hydrolysis of spirohydantoin mustard. J. Pharm. Sci. 71: 1206-1211, 1982.

3. Cooney, D.A., Jayaram, H.N., Gebeyehu, G., Betts, C.R., Kelley, J.A., Marquez, V.E. and Johns, D.G.: The conversion of 2-β-D-ribofuranosylthiazole-4-carboxamide to an analogue of NAD with potent IMP dehydrogenase-inhibitory properties. Biochem. Pharmacol. 31: 2133-2136, 1982.

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5. Gebeyehu, G., Marquez, V.E., Kelley, J.A., Cooney, D.A., Jayaram, H.N. and Johns, D.G.: Synthesis of thiazole-4-carboxamide-adenine dinucleotide (TAD). A powerful inhibitor of IMP-dehydrogenase. J. Med. Chem. (in press).

6. Cooney, D.A., Jayaram, H.N., Glazer, R.I., Kelley, J.A., Marquez, V.E., Gebeyehu, G., Van Cott, A., Zwelling, L.A., Johns, D.G.: Studies on the mechanism of action of tiazofurin. Metabolism to an analog of NAD with potent IMP dehydrogenase-inhibitory activity. Adv. Enz. Reg., (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM06152-01 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Biochemical Toxicology of Anthracyclines; the Role of Reactive Oxyradicals.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Edward G. Mimnaugh, B.A., Chemist, LMCP, NCI

COOPERATING UNITS *(if any)*

Dept. of Pharmacology, George Washington University, Washington, D.C.

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Interactions Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cardiotoxic effects of anthracyclines are well documented, and a growing body of experimental evidence has implicated reactive forms of oxygen in this life-threatening toxicity. Adriamycin and other anthracycline anticancer drugs can be enzymatically activated to semiquinone free radical intermediates which autoxidize to generate superoxide anion radical and other highly reactive and toxic oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen. These oxyradicals and activated species of oxygen can cause toxicity by attacking and damaging intracellular target molecules including nucleic acids, structural proteins, enzymes and especially, membrane unsaturated lipids. Reactive oxygen attack of membrane lipids causes extensive damage by the process of membrane lipid peroxidation, which not only disrupts the structural integrity of the membrane, but also inactivates membrane bound enzymes and produces toxic, reactive aldehyde products which can alkylate proteins and nucleic acids. Thus, anthracycline-enhanced membrane lipid peroxidation may cause damage by both direct and by secondary mechanisms. The present projects were designed to evaluate this hypothesis and to better understand the possible biochemical and molecular mechanisms which contribute to anthracycline cardiac toxicity.

PHS 6040 (Rev. 1/83) GPO 895-100

Other Investigators:

Theodore Gram, Ph.D.
Michael Trush, Ph.D.
Birandra Sinha, Ph.D.
Katherine Kennedy, Ph.D.
Edward Acton, Ph.D.

1. Enhancement of Rat Heart Microsomal Lipid Peroxidation Following Doxorubicin Treatment In Vivo. Although cardiac microsomes from α-tocopherol deficient rats were susceptible to greatly enhanced microsomal lipid peroxidation in the presence of NADPH and doxorubicin, in contrast, heart microsomes from control animals were refractory to doxorubicin-enhanced lipid peroxidation. Interestingly, cardiac microsomes from rats treated with doxorubicin were susceptible to lipid peroxidation which was enhanced more than 15-fold by doxorubicin in vitro. These results indicate that doxorubicin treatment in vivo caused biochemical alterations, possibly a transient decrease in the membrane antioxidant, α-tocopherol, which predisposed cardiac microsomes to anthracycline free radical-generated oxyradical attack and lipid peroxidation.

2. Possible Role for Membrane Lipid Peroxidation in Anthracycline Nephrotoxicity. Reactive oxyradicals produced during the enzymatic redox cycling of anthracyclines may be responsible for the damage caused by anthracyclines in kidney. Adriamycin accentuated kidney microsomal NADPH-dependent lipid peroxidation (LP) by 7-fold. Appreciable NADH-dependent LP was also measured in kidney microsomes, suggesting that NADH-cytochrome b_5 reductase, as well as NADPH-cytochrome P-450 reductase, may have participated in the metabolic activation of adriamycin. In kidney mitochondria, adriamycin produced a 14-fold increase in NADH-supported LP, resulting from the interaction of adriamycin with mitochondrial NADH-dehydrogenase. These results show that metabolic activation of adriamycin by kidney microsomal and mitochondrial enzymes results in extensive oxyradical-mediated membrane lipid peroxidation which may be responsible for the observed nephrotoxicity of this drug.

3. Adriamycin-accentuated Membrane Lipid Peroxidation in Isolated Nuclei. The production of toxic oxyradicals by adriamycin could potentially cause extensive damage to DNA, however, it is also possible that peroxidation of nuclear envelope membranes could contribute to the morphologic and biochemical alterations observed in the nucleus following adriamycin treatment. Adriamycin (ADR) caused a 6 to 8-fold enhancement of endogenous NADPH-dependent nuclear lipid peroxidation (LP) which was maximal at 300 μM ADR. NADH also supported ADR-accentuated nuclear membrane LP, although to a lesser extent than NADPH. Superoxide dismutase, 1,3-dimethylurea, and reduced glutathione, inhibited ADR-stimulated LP in isolated nuclei indicating that oxyradicals were involved. DETPAC was also inhibitory, suggesting that metal ions had an important role in the peroxidation reactions. By adding ascorbate and exogenous iron the maximum peroxidation of lipids was obtained, and it was determined that within 2 hours approximately half of the unsaturated nuclear membrane lipids were peroxidized by NADPH-supported, adriamycin-dependent oxyradical production. These results suggest that anthracycline-enhanced lipid peroxidation of the nuclear membrane may have an important role in the toxicity of anthracyclines.

4. Inhibition of Doxorubicin-Stimulated Cardiac Sarcosomal Membrane Lipid Peroxidation by Other Anticancer Agents.
Usually anticancer agents are selected for combination chemotherapy of neoplastic disease on the basis that they have different mechanisms of action and minimal overlapping toxicities. Given that accumulating evidence suggests that doxorubicin cardiotoxicity may result from membrane lipid peroxidation, appreciable therapeutic benefit could be obtained if other anticancer drugs could block doxorubicin-stimulated membrane lipid peroxidation. In this context, the drugs, 5-iminodaunorubicin, mitoxantrone, 9,10-aminoanthracenedione, and bleomycin all inhibited adriamycin-enhanced cardiac sarcosomal membrane lipid peroxidation (LP). For all 4 agents the apparent K_I values were 8 to 10 μM. These drugs may decrease peroxidation by preventing or limiting the enzymatic activation of adriamycin, or alternatively, by chelating trace amounts of iron that are necessary for LP. An evaluation of the possibility that these drugs may lessen the cardiotoxicity of adriamycin in vivo is currently in progress.

5. Enhancement of NADPH-Dependent Cardiac Microsomal Membrane Lipid Peroxidation by Several New Anthracycline Antitumor Drugs: Comparison with Adriamycin and Daunorubicin. Many anthracycline anticancer agents have the capability of enhancing microsomal lipid peroxidation (LP) several fold. Interestingly, other structurally related agents, including 5-iminodaunorubicin and aminoanthracenediones, inhibit peroxidation. Several new anthracycline drugs, 4-Morpholinyldaunorubicin, cyanomorpholinyldaunorubicin and 4-methoxypiperidinyldaunorubicin, all enhanced NADPH-dependent mouse heart microsomal LP in a concentration-dependent fashion. At equivalent concentrations each analog was equipotent to daunorubicin and twice as potent as adriamycin. The modifications of the sugar moiety of daunorubicin found in the three compounds apparently have little or no effect on the ability of the analogs to stimulate membrane LP. Thus, these new drugs may cause cardiotoxicity in a manner similar to adriamycin and daunorubicin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CM07119-04 LMCB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Biochemical Toxicology of Oncolytic Platinum Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Charles L. Litterst, Ph.D., Pharmacologist, LMCB, NCI

COOPERATING UNITS *(if any)*
1. Laboratory of Experimental Pathology, DCCP, NCI
2. Kresge Hearing Research Institute, University of Michigan

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Interactions Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the toxic effects of cisplatinum on the kidney have been appreciated for some time, the renal handling of cisplatinum and the mechanism by which the renal toxicity occurs are still incompletely understood. These mechanisms could be more easily defined if the molecular sites of interaction of cisplatin were recognized. This project is designed to define how the kidney handles cisplatin under normal condtions and after various pretreatment or other experimental conditions. Inherent in this study is an attempt to localize the sites of interaction of cisplatin and its intracellular binding sites. This section reports the definitive animal study on renal handling of cisplatin, the effect of multiple treatments on renal handling of cisplatin, effect of changes in metabolic pH on cisplatin toxicity and distribution, studies that presumptively identify 2 low molecular weight binding sites for cisplatin, and interactions between cisplatin and drug metabolizing enzymes.

Other Investigators:

Federico Bertolero, M.D.
Vanessa Schweitzer, M.D.
Nahed Osman, M.D., Ph.D.
Zahid Siddik, Ph.D.
Yochiro Hirokata, M.D., Ph.D.
Samuel Tong, Ph.D.

1. Interactions between cisplatinum and possible tissue receptors. At 3, 5, 8 days after treatment with cisplatinum hepatic cytochrome P-450 dependent enzyme activity was increased but levels of cytochromes P-450 and b_5 were unaltered. Hepatic lipid peroxidation (LP) was increased at all times, with a maximum of 20 times normal. Renal aniline hydroxylase was elevated throughout the first 8 days. Renal LP was not significantly altered. Hepatic levels of glutathione were depressed at 3, 5 days after dosing. Renal levels of glutathione were increased by 3-5 fold at 8 and 12 days after drug administration and were decreased at all other times. Activity of s-aryl transferase was increased and S-epoxidetransferase was decreased at 5, 8, 12 days after dosing. No effects on enzyme activity were observed when cis-Pt was added in vitro. When cisplatin and reduced glutathione were determined chromatographically in tissue cytosols from treated animals, 30% of the recovered platinum was associated with glutathione. The elution profile of radioactive platinum from liver & kidney cytosols from cisplatin-treated animals showed radioactivity mainly in high molecular weight (HMW) regions but with peaks in low molecular weight (LMW) regions also. One LMW peak was present in liver and kidney at all times studied. Binding of platinum to this peak increased in rats that had been pretreated with $CdCl_2$, but not in rats pretreated with cis-Pt. This component has some of the chromatographic characteristics of metallothionein (MT). Platinum was also associated with a chromatographic peak that was positive when tested for free sulfhydryl groups and had a molecular weight less than 1000.

2. Mechanism of toxicity and renal handling of cisplatin. In animals pretreated with cisplatinum for various times before administration of ^{195m}Pt-labelled cisplatinum, concentrations of ^{195m}platinum were greater in tissues and in tissue subcellular fraction of pretreated animals than in controls. BUN was elevated on day 1 only in those animals which had been pretreated. Urinary excretion of platinum was less in pretreated than in control animals. Pretreatment with cisplatinum may damage the kidney so severely that subsequent doses are not excreted as efficiently. The renal clearance of free cisplatin was the same as GFR. Probenecid resulted in a 70% increase in GFR but N-methylnicotinamide did not affect GFR. Cisplatinthus is excreted by a process which includes active reabsorption via the organic acid transport system. Ammonium chloride (AC)-pretreated rats did not exhibit the characteristic cisplatin-induced diuresis and were unable to maintain an acidic urinary pH. AC-pretreated rats had a lower and sodium-bicarbonate (SB) pretreated rats a higher urinary excretion of Pt than did controls. Platinum excretion was correlated with urinary pH and not urinary volume. The renal concentration of platinum was greater in AC animals than in SB or control animals. Both pretreated groups had equal percent of free vs bound platinum. Proteinuria was

more severe in AC-pretreated rats, but histologic evidence of tubular damage was present in all groups. Enhanced Pt concentrations were observed in ovaries and uterus after cisplatin was administered into the common iliac artery. A therapeutic advantage may be gained by regional IA administration of cis-Pt for pelvic neoplasms.

Pharmacokinetics of Experimental drugs: Analogs of Cisplatin. Plasma decay curves of the cisplatin analog CBDCA showed little protein binding and almost total clearance from the plasma within 4 hours. Blood clearance had half times of 13 minutes and 15 hours. By 4 hours after treatment 100% of the dose could be recovered in urine. Platinum concentrations were highest in kidney, with high concentrations also found in liver and skin.

Publications

1. Litterst, C.L., Tong, S., Hirokata, Y., and Siddik, Z.H.: Alterations in hepatic and renal levels of glutathione and activities of glutathione S-transferases from rats treated with cis-dichlorodiammineplatinum (II). Cancer Chemother. Pharmacol. 8: 67-71, 1982.

2. Litterst, C.L., Sieber, S.M., Copley, M., and Parker, R.J.: Toxicity of free and liposome-encapsulated adriamycin following large volume, short term intraperitoneal exposure in the rat. Toxicol. Appl. Pharmacol. 64: 517-528, 1982.

3. Bonnem, E.M., Litterst, C.L., and Smith, F.P.: Platinum concentrations in human glioblastoma multiforme following the use of cisplatinum. Cancer Treat. Rep. 66: 1661-1663, 1982.

4. Bertolero, F., and Litterst, C.L.: Changes in renal handling of platinum in cisplatinum-treated rats following induction of metabolic acidosis or alkalosis. Res. Comm. Chem. Path. Pharmacol. 36: 273-285, 1982.

5. Litterst, C.L.: Cisplatinum: A review, with special reference to cellular and molecular interactions. Ann. Clin. Lab. Sci. in press, 1983.

6. Litterst, C.L., Tong, S., Hirokata, Y. and Siddik, Z.: Stimulation of microsomal drug oxidation in liver and kidney of rats treated with the oncolytic agent cis-dichlorodiammineplatinum (II). Pharmacology 26: 46-53, 1982.

7. Schweitzer, V.G., Hawkins, J.E., Lilly, D.J., Litterst, C.L., Abrams, G., Davis, J.A., Christy, M: Ototoxic and nephrotoxic effects of combined treatment with cisdiamminedichloroplatinum and kanamycin in the guinea pig. Otolaryn. Head Neck Surg. in press, 1983.

8. Litterst, C.L., Flora, K.P., Cradock, J.C.: Bioavailability of Δ^9-11-^{14}C-tetrahydrocannabinol to rabbits. Res. Commun. Substances Abuse 3: 453-465, 1982.

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10. Litterst, C.L. and Schweitzer, V.G.: Increased tissue deposition and decreased excretion of platinum following administration of cisplatinum to cisplatinum-pretreated animals. Cancer Chemother. Pharmacol. in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM07120-04 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Role of Drug Metabolism in Modulating Toxicological Responses.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Theodore E. Gram, Ph.D., Pharmacologist, LMCB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Drug Interactions Section

INSTITUTE AND LOCATION

National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.8	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The existence and biochemical mechanisms of organ-specific toxicity are the subject of heightened interest among toxicologists. Earlier studies in this laboratory described selective necrosis of pulmonary non-ciliated bronchiolar (Clara) cells following administration of naphthalene to mice. No damage to other lung cells was noted and no pathologic changes, as evidenced by histology or enzymic alterations, were observed. The work described in this section describes conditions under which 1,1-dichloroethylene (DCE) produces selective damage to mouse lung without morphologic or enzymatic evidence of nephro- or hepatotoxicity.

Other Investigators:

Klaas Krijgsheld, Ph.D.
Michael Trush, Ph.D.
Yoichiro Hirokata, M.D., Ph.D.
Edward Mimnaugh, B.S.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

Damage to Clara Cells and Inhibition of Pulmonary Mixed-Function Oxidation by 1,1-dichloroethylene.

A single dose of 1,1-dichloroethylene (DCE) injected i.p. into mice caused a reduction of cytochrome P-450 and monooxygenases in lung microsomes with no corresponding changes in liver and kidney. Light microscopy revealed necrosis restricted to the Clara cells. Liver and kidney were relatively unaffected by DCE treatment. Maximal inhibition of pulmonary monooxygenases occurred at 2-4 days and returned to normal about day 21. Cytochrome P-450, NAPDH cytochrome c reductase, benzphetamine N-demethylase, and ethoxyresorufin-O-deethylase activities were 50% of control levels while AHH activity was less severely affected. Coumarin 7-hydroxylase was 10% of control 4 days after DCE. The slow return of normal enzyme activities was paralleled by a correspondingly slow reappearance of bronchiolar Clara cells.

Publications

1. Tong, S.S., Lowe, M.C., Trush, M.A., Mimnaugh, E.G., Ginsburg, E., Hirokata, Y. and Gram, T.E.: Bronchiolar epithelial damage and impairment of pulmonary microsomal monooxygenase activity in mice by naphthalene. Exp. Mol. Pathol. 37: 358-369, 1982.

2. Krijgsheld, K.R., Lowe, M.C., Mimnaugh, E.G., Trush, M.A., Ginsburg, E. and Gram, T.E.: Lung-selective impairment of cytochrome P-450-dependent monooxygenases and cellular injury by 1, 1-dichloroethylene in mice. Biochem. Biophys. Res. Commun. 110: 675-681, 1983.

3. Gram, T.E.: The pulmonary mixed function oxidase system. In Witschi, H.P. and Brain, J.D. (Eds): The Toxicology of Inhaled Materials. Part I: General principles of inhalation toxicology, Berlin, Springer-Verlag, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CM07121-04 LMCB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Involvement of Reactive Forms of Oxygen in Drug-Induced Pulmonary Toxicity.

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Michael A. Trush, Ph.D., Sr. Staff Fellow, LMCB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Medicinal Chemistry and Biology

SECTION
Drug Interactions Section

INSTITUTE AND LOCATION
National Cancer Institute, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Life-threatening pulmonary toxicity as a result of anticancer drug therapy is becoming increasingly recognized. It is also becoming apparent that because of the inherent molecular properties of some antineoplastic agents, reactive oxygen may be involved in the cytotoxic reaction(s) to lung cells. The drug-induced generation of reactive forms of oxygen (superoxide anion, hydroxyl radical and singlet oxygen) can contribute to drug cytotoxicity through attack of reactive oxygen species on intracellular targets (nucleic acids, lipids, proteins) and/or through reactive oxygen-mediated activation of the drug to an active intermediate. The present projects were designed to evaluate these hypotheses in order to better understand the possible biochemical and molecular mechanisms which contribute to pulmonary toxicity elicited by antineoplastics.

Other Investigators:

Theodore Gram, Ph.D.
Edward Mimnaugh, B.S.
Erika Ginsburg, B.S.

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

1. Study of the Ability of Misonidazole and 4-Nitroquinoline N-Oxide to Stimulate Reactive Oxygen-Dependent Lipid Peroxidation in Mouse Lung Microsomes. Misonidazole is a nitro-containing drug currently being utilized to increase the sensitivity of hypoxic tumor cells to ionizing radiation. 4-Nitroquinoline N-oxide (4NQO) administration induces pulmonary tumors and leukemia in rodents. Both of these compounds are capable of being metabolically activated to a nitro anion radical intermediate which, under aerobic conditions, autoxides and generates reactive oxygen species capable of damaging biomolecules such as lipids and DNA. This project examines the ability of these compounds to enhance lipid peroxidation in microsomes isolated from mouse lung. A significant enhancement in lipid peroxidation was observed at 10 μM 4-NQO whereas 5 mM misonidazole was required in order to significantly enhance this reaction. The augmentation of lipid peroxidation by these two compounds was inhibited by superoxide dismutase, demonstrating the reactive oxygen dependency of this reaction. Thus, it is possible that reactive oxygen metabolites and/or toxic products of lipid peroxidation are involved in the induction of pulmonary tumors by 4-NQO. On the other hand, it is unlikely that pulmonary toxicity will be a significant consequence of misonidazole administration.

2. Factors which Affect the Microsomal "Activation-Inactivation" of Bleomycin: The Role of Superoxide Anion. Incubation of bleomycin with rat lung microsomes in the absence of DNA results in a time-dependent decline in the ability of bleomycin to inhibit lipid peroxidation and mediate DNA damage. This microsome-catalyzed inactivation of bleomycin was found to be dependent on both oxygen and NADPH, suggesting that a similar mechanism may be involved in bleomycin-mediated DNA damage and bleomycin inactivation. Based on this conclusion, the hypothesis was examined that any factor which increases bleomycin-mediated DNA damage should similarly increase bleomycin inactivation. As anticipated, the addition of Fe^{3+}, ascorbic acid and the redox cycling compound, paraquat increased both bleomycin-mediated DNA damage and bleomycin inactivation. The enhanced inactivation of bleomycin by these agents is oxygen dependent. Superoxide dismutase and EDTA inhibited the inactivation of bleomycin, suggesting the involvement of adventitious iron and superoxide anion in this process. These results suggest that superoxide probably derived from microsomal NADPH cytochrome P-450 reductase, mediates the reduction of bleomycin-bound Fe^{3+} to Fe^{2+} which, in turn, facilitates the activation of BLM to a DNA damaging species.

3. Bleomycin-Mediated Deoxyribose Cleavage in Rabbit Lung Nuclei. The NADPH-dependent pulmonary microsomal mixed-function oxidase (MFO)-catalyzed activation of bleomycin (BLM) results in deoxyribose cleavage of exogenously added DNA.

Inasmuch as the MFO system associated with the nuclear membrane is capable of metabolically activating xenobiotics, we have examined the interaction of BLM with nuclei isolated from rabbit lung to determine if DNA damage resulted. In the presence of NADPH, a 6-fold increase was observed in thiobarbituric acid reactive products (TBAR) indicative of BLM-mediated doexyribose cleavage. Fe^{3+} or ascorbic acid significantly enhanced this reaction while GSH was inhibitory. These agents also exert a similar modulatory influence on BLM-mediated DNA damage catalyzed by lung microsomes, indicating that the mechanism for bleomycin activation and accompanying deoxyribose cleavage is similar between the two subcellular fractions. Moreover, the inclusion of lung microsomes enhanced BLM-elicited TBAR from nuclear DNA 4-fold. Thus, these results demonstrate that BLM activated by either the microsomal or nuclear membrane MFO system is capable of damaging DNA within intact nuclei and, as such, could contribute to the pulmonary cytotoxicity of BLM.

4. Characterization of the Interaction of Bleomycin with Microsomes Isolated from Lewis Lung and B16 Melanoma. DNA is generally considered the cellular target by which bleomycin elicits cytotoxicity and exerts its antiproliferative activity. In order for bleomycin to damage DNA it is necessary for it to be activated. Since the mixed-function oxidase system represents an efficient biological mechanism for the activation of bleomycin, we examined the ability of microsomes isolated from the Lewis lung carcinoma and the B16 melanoma to catalyze bleomycin-mediated DNA deoxyribose cleavage. Microsomes from both tumors were able to catalyze this reaction although those from the B16 melanoma exhibited three times the activity as microsomes isolated from the Lewis lung carcinoma. The addition of ascorbic acid, Fe^{3+} or the redox cycling chemical, paraquat, significantly enhanced this reaction whereas EDTA, superoxide dismutase and glutathione inhibited the cleavage of deoxyribose by bleomycin. In addition to bleomycin A_2, bleomycn PYP (NSC 276381), bleomycin BAPP (NSC 294979), bleomycin PEP (NSC 276382), and tallysomycin A (NSC 279496) were activated to a DNA damaging species by microsomes from the B16 melanoma and the Lewis lung carcinoma. It is hoped that future studies planned on the in vitro interaction of bleomycin with tumor microsomes will lead to a better understanding of mechanisms by which to increase the therapeutic effectiveness of this drug.

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5. Trush, M.A.: Studies on the interaction of bleomycin A_2 with rat lung microsomes. III. Effect of exogenous iron of bleomycin-mediated DNA chain breakage. Chem. Biol. Interact. in press (1983).

6. Kensler, T.W. and Trush, M.A.: Inhibition of oxygen radical metabolism in phorbol ester-activated polymorphonuclear leukocytes by an antitumor promoting copper complex with superoxide dismutase-mimetic activity. Biochem. Pharmacol. in press, 1983.

7. Trush, M.A., Reasor, M.J., and Van Dyke, K.: Oxidant-Mediated Electronic Excitation of Imipramine. Biochem. Pharmacol. in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM07129-02 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Copper and Its Chelates in Cytotoxicity and Chemotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Marco Rabinovitz, Head, Molecular Biology and Methods Dev. Section, LMCB, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Medicinal Chemistry and Biology

SECTION

Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	0.8	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Murine L1210 cells in primary culture in common with a wide range of human tumor cells in primary culture require mercaptoethanol or other thiols for growth. We have observed that the copper-specific chelator, bathocuproine sulfonate, could also support the growth of L1210 cells in primary culture. By removing available copper ions normally present in the tissue culture medium, it retarded the oxidation of cysteine to poorly utilized cystine. Evidence that copper ion was inhibitory only by functioning as an extracellular catalyst for oxidation of cysteine was supported by the observation that the mixed disulfide of methyl mercaptan and cysteine, 4-thiamethionine, supported good growth of the cells in primary culture even in the presence of of 100 μM copper sulfate. 4-Thiamethionine is transported into the cells by the same mechanism as is methionine but is reduced to methyl mercaptan and cysteine inside the cell. It therefore permits the cell to grow where cystine transport is ineffective and where cysteine is rapidly oxidized to the non-utilized cystine.

Other Investigators:

Anish Mohindru	Visiting Fellow (until 11/15/82)	LMCB	NCI
Herbert Pierson	Guest Worker, PRAT Fellow (from 7/1/83)	LMCB	NCI
Joyce Fisher	Chemist	LMCB	NCI

Objectives:

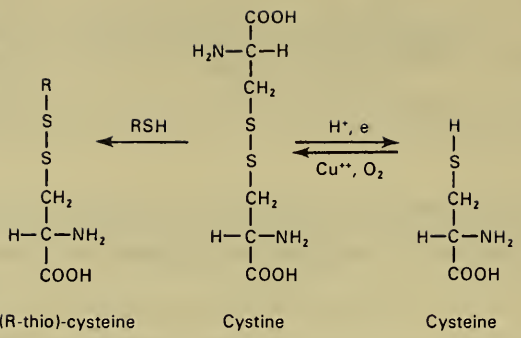
Our studies are directed toward understanding the basis of copper-mediated cytotoxicity and the sensitivity to copper and its chelates by tumor cells. This information is applied to the design of regimens for chemotherapy.

Methods Employed:

The principal methods employed involve culture techniques such as primary and established cell culture, viability estimates by clonal growth, and cell size distribution as well as chemotherapeutic evaluation with tumor bearing mice. Studies with isotopically labelled components are also carried out in experiments designed to ascertain mechanisms of action.

Major Findings:

1. 'Oxidative Damage' to a Lymphoma in Primary Culture under Aerobic Conditions is due Solely to a Nutritional Deficiency of Cysteine. The mercaptoethanol requirement for growth of L1210 cells in primary culture can be met by the addition of bathocuproine disulfonic acid (BCS), a copper specific chelating agent. BCS reduces the rate of oxidation of cysteine to cystine in the tissue culture media, a reaction long recognized to be catalyzed by the endogenous copper of serum. With the rate of cysteine reoxidation reduced, growth is markedly stimulated by trace reductants released into the medium which convert non-utilizable cystine to utilizable cysteine. These reductants may be oxidized and intercepted by Cu^{++} or the poorly permeable disulfide, 6,6'dithiodinicotinic acid, added to a concentration of 50 μM. However, the requirement for mercaptoethanol, cysteine or a reduced Cu^{++} level can be met by the addition of S-(methylthio)-L-cysteine, which may be considered a methionine analog, 4-thiamethionine. This compound is taken up by the cells via the leucine transport system, and reduced intracellularly to cysteine to satisfy the requirement for this amino acid. It is active at low concentrations where added cysteine or dimethyldisulfide are inactive. In the presence of 4-thiamethionine, the in vivo derived L1210 cells, normally inhibited in culture by trace endogenous copper, grow well in the presence of 0.1 mM added copper sulfate, like the established L1210 line. Such growth under oxidative stress indicates that the cells in primary culture are not principally damaged by oxygen-derived free radicals or peroxide, but first suffer the deprivation of an essential amino acid, cysteine. The relationship between thiol requirement and growth inhibition by trace copper is illustrated by the equations below.



2. Copper Chelates of the 1,10-Phenanthroline Series are Toxic to Tumor and Host by Different Mechanisms. 2,9-Dimethyl-1,10-phenanthroline, a copper-specific chelator, is a potent cytotoxin to L1210 murine leukemia cells in vitro. Its activity was dependent upon available Cu^{+2} in the medium and other divalent ions, Fe^{+2} and Zn^{+2}, were ineffective as promotors of cytotoxicity. As the copper chelate it produced a 4 log kill at 4 μM after a 1 hr incubation. This was in marked contrast to its isomer, 4,7-dimethyl-1,10-phenanthroline, which also inhibited cell growth, but whose inhibition was overcome by added Cu^{+2}. Both the 2,9- and 4,7-dimethyl isomers, however, were toxic to mice when injected in the presence, but not in the absence of copper. These divergent results indicate that these copper chelates function by different mechanisms in their toxicity to tumor and host.

Proposed Course:

Combined Copper-Delivery Agents in Cancer Chemotherapy. Since 2,9-dimethyl-1,10-phenanthroline had significant activity against the P388 murine leukemia (last year's report) and appears to have host toxicity not identical in mechanism to that which it directs against the tumor, it will be combined at reduced levels with other ligands which are toxic in the presence of copper in order to minimize host toxicity and maximize copper toxicity to the tumor.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CM07130-01 LMCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mercaptan and Disulfide Potentiators of Diselenide Toxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Marco Rabinovitz, Head, Molecular Biology and Methods Dev. Section, LMCB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Medicinal Chemistry and Biology

SECTION

Molecular Biology and Methods Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dimethyldisulfide reduced the effective concentration of selenocystamine for cytotoxicity to L1210 murine leukemia cells in culture by two orders of magnitude. It also elicited a marked increase in the toxicity of selenocystine. The results are interpreted as due to an improved uptake of the selenium compounds via the mixed selenosulfides. This is supported by the observation that the toxicity of selenocystine promoted by dimethyldisulfide was reduced in the presence of a high concentration of leucine. This indicates that the selenosulfide adduct, 4-selenamethionine, was taken up by the cell via the leucine-preferring transport system. The specificity of the disulfide for promotion of selenocystine toxicity is more rigorous than that for promotion of growth under conditions of limiting cystine availability. Under the latter conditions, the oxidized form of mercaptoethanol, hydroxyethyldisulfide, was highly effective, but under the former it was a poor potentiator of toxicity. At present we do not know whether this difference is due to the rate of thiol-selenide interaction or to uptake by the cell of the selenosulfide compound.

Cells inhibited in growth by the selenosulfide adducts were still viable when washed free of the inhibitor after incubation for 8 hours. The mechanism of inhibition and possible differences in specificity of the disulfide among various cell types will be investigated.

The goal of this project is to provide a fundamental understanding of factors promoting selenide uptake and to apply this information for use of diselenides in cancer chemotherapy.

Other Investigator:
Joyce Fisher Chemist LMCB NCI

Objectives:

To determine factors determining the transport, cytotoxicity and specificity of organic selenides, with the view of evaluating their use as chemotherapeutic agents.

Methods Employed:

The principal methods employed involve culture techniques such as primary and established cell culture, viability estimates by clonal growth, and cell size distribution as well as chemotherapeutic evaluation with tumor bearing mice. Studies with isotopically labelled components are also carried out in experiments designed to ascertain mechanisms of action.

Major Findings:

In view of the role of low molecular weight disulfides in the promotion of cystine uptake for growth, we investigated their effectiveness in promoting selenocystine and selenocystamine uptake for cytotoxicity. Dimethyldisulfide reduced the effective concentration of selenocystamine for cytotoxicity by 2 orders of magnitude and also elicited a marked increase in the toxicity of selenocystine. This toxicity of selenocystine promoted by dimethyldisulfide was reduced in the presence of a high concentration of leucine, indicating that the selenosulfide adduct, 4-selenamethionine, was taken up by the cell via the leucine-preferring transport system. The cells were still viable when growth inhibition was established for eight hours, but prolonged incubation of twenty hours and more resulted in cell death.

A higher degree of specificity for the structure of the low molecular weight disulfide was observed in promotion of diselenide cytotoxicity than in promotion of cell growth under conditions of limiting cystine availability. Thus hyroxyethyl disulfide, a good promotor of cell growth under conditions of limiting cystine availability, was a poor promotor of diselenide toxicity. Cystamine was ineffective in promoting selenocystamine toxicity at at 250:1 molar ratio, and at this high molar ratio, did not compete with selenocystamine for dimethyldisulfide. The mixture of selenocystamine and selenocystine was not synergistic at toxic levels of each. These observations indicate a limiting range of specificity for active disulfides or mercaptans.

Proposed Course:

At present it is not clear to what extent selenol or diselenide analogs of sulfur metabolites act as antimetabolites or are non-specific poisons. They may compete for transport systems but react in a non-specific manner inside the cell. By altering the routes of transport via selenosulfide adducts in cells under different states of cystine supplementation, it should be possible to resolve this matter for selenocystine. We also wish to investigate possible in vivo implications of the selenosulfide adducts for tumor and host toxicity

and, if possible, as a structure-activity relationship of the disulfide moiety.

Publications:

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2. Naujokaitis, S.A., Fisher, J.M. and Rabinovitz, M.: Tetraalkylammonium ions: Protection of murine L1210 leukemia and bone marrow progenitor cells in vitro against mechlorethamine cytotoxicity and inhibition of the choline transport system. Chem. Biol. Interact. 40: 133-140, 1982.

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6. Mohindru, A., Fisher, J.M. and Rabinovitz, M.: 2,9-Dimethyl-1,10-Phenanthroline (Neocuproine): A potent, copper-dependent cytotoxin with anti-tumor activity. Biochem. Pharmacol., in press.

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

The Laboratory of Molecular Pharmacology is engaged in studies to determine the molecular mechanisms of action of anti-cancer drugs. Much of the work concerns bifunctional alkylating agents, DNA intercalating agents, and related drugs. The major questions are: What DNA lesions or other effects on DNA are produced by these drugs. To what extent are specific DNA lesions repaired in various cells. How do the effects on DNA relate to cytotoxicity? Of particular interest are differences depending on cell type that give rise to differential cytotoxicity which may provide a selective action against susceptible human neoplasms. Studies are also being carried out on the functional regulation of histone proteins, in order to learn more about the control of the cell cycle and of cell differentiation. In this context, we are studying the action of some cell cycle-dependent anti-cancer drugs.

Mechanism of Action of Chloroethylnitrosoureas (CNUs).

We had previously found that human tumor cell strains generally fall into two groups which differ in the formation of chloroethylnitrosourea (CNU)-induced interstrand crosslinks (ISCs). The strains that generated high frequencies of CNU-induced ISCs, were deficient in DNA guanine-O^6-methyl transferase activity, a phenotype that is designated Mer^-. The Mer^- strains had an increased sensitivity to CNU-induced cell killing, thus indicating that the ISCs, or associated lesions, are important in the killing of human tumor cells. We had proposed the hypothesis that, among the DNA alkylation products produced by CNUs, there are guanine-O^6 chloroethylations which can slowly react with the opposite DNA strand to form ISCs, and that these DNA-chloroethyl monoadducts are rapidly removed by the guanine-O^6-alkyl transferase activity in Mer^+ tumor cells (as well as in normal human cells) thereby preventing ISC formation and enhancing cell survival.

During the past year, we have further tested this hypothesis by the use of DNA methylating agents which inactivate the guanine-O^6-alkyl transferase. It was found that pretreatment of Mer^+ tumor cells or normal human cells with methylating agents increased CNU-induced ISC formation from undetectable to easily measurable levels. The pre-treatment also sensitized these cells to CNU-induced killing. The results were thus in accord with our hypothesized mechanism and supported the importance of ISC formation in the killing of human tumor cells by CNUs.

We plan to continue the study of this mechanism, using an assay for potentially crosslinkable DNA monoadducts which we have just developed. We are also beginning to use HPLC assays to study the formation and repair of CNU-induced DNA adducts.

Carbamoylating Activity.

The carbamoylating activity of CNUs is not required for antitumor activity, nor

does it by itself show antitumor activity. We had previously found that carbamoylation increases the cytotoxicity of CNUs to both normal and malignant human cells, thereby reducing the differential cytotoxicity to Mer^- tumor cells relative to Mer^+ normal cells. Our evidence suggested that carbamoylating activity would reduce the potential effectiveness of CNUs against human tumors by interfering with a DNA repair system which normal human cells need in order to withstand CNU treatment.

During the current year we have tested this hypothesized mechanism, using normal human cells that do not show detectable CNU-induced ISCs. The absence of ISC formation in these cells facilitated the study of other sources of cytotoxicity. We found that CNUs having strong carbamoylating activities markedly inhibit the repair of X-ray-induced strand breaks in these cells, whereas CNUs that lack carbamoylating activity do not inhibit this repair. Strong carbamoylating CNUs exerted clearly greater cytotoxicity on these normal Mer^+ cells than did non-carbamoylating CNUs. The cytotoxicity against Mer^- tumor cells, on the other hand, was not affected by carbamoylating activity. These results, together with our previous findings, convince us that CNUs currently in clinical use should be replaced with non-carbamoylating derivatives.

Mechanism of Action of Aziridinylbenzoquinone (AZQ).

The effects of this clinically promising drug on DNA in cells and subcellular systems were studied, and new insights into its mechanism of action were obtained. The chemical structure of the drug suggests that it could have two types of reactivity: (1) oxidation-reduction activity which could lead to free-radical reactions, and (2) bifunctional alkylation activity which could generate cross-links. In accordance with this expectation, AZQ was found to generate (1) DNA strand breaks in an NADPH-requiring and superoxide dismutase-inhibitable reaction and (2) DNA interstrand crosslinks (ISCs) by means of an alkylation reaction which was greatly enhanced by reduction of the AZQ quinone. The enhanced alkylation and enhanced ISC formation by reduced AZQ however was unexpected. These findings suggest that, contrary to the generally accepted view, AZQ may be a bio-reductive alkylating agent and might be useful in the treatment of hypoxic tumor tissue.

The extents of the two reactions of AZQ, as signalled by the formation of strand breaks and ISCs, respectively, was found to vary greatly and independently in different cell lines. The most striking differences were between a human colon tumor line (HT-29) which exhibited high ISC levels with little or no strand breakage, and normal human cells (IMR-90) which exhibited extensive strand breakage without detectable ISC formation. Cytotoxicity was more closely correlated with ISC formation than with strand breakage. The large differences in DNA lesions among different human cell types encourages the hope that large selective effects against suitably selected human tumors could be achieved.

Intercalator Effects on DNA: Molecular Mechanism.

Our previous work had led to the hypothesis that DNA intercalating agents produce protein-associated DNA strand breaks by the following mechanism: (1) intercalation reduces the twist of the DNA helix, (2) the change in helix twist produces torsional strain which is propagated some distance along the helix, (3) the torsional strain brings into action a topoisomerase-like enzyme which introduces strand breaks and becomes covalently bound to one terminus of the strand

break, (4) the introduced strand breaks allow the winding number of the helix to change so as to relieve the torsional strain. We had obtained evidence to support the enzymatic nature of the effect. During the past year, we have put this hypothesis to further experimental tests using the intercalating agent, m-AMSA. The results have caused us to generate a new and, we think, exciting picture of what may be happening.

The first question addressed was whether protein-associated strand breaks allow the strand break termini to swivel so as to change the winding number. Using the nucleoid sedimentation technique, it was unexpectedly found that, at least under the high-salt conditions of this assay, swivelling does not occur unless a proteolytic treatment is introduced. This result cast doubt on the hypothesized role of a topoisomerase-like enzyme in relieving intercalator-induced torsional strain.

The second question was whether the protein-associated strand breaks are formed as a response to torsional strain. If this were true then the introduction of a sufficient number of single-strand breaks, for example by x-ray, would prevent the accumulation of torsional strain in response to intercalator and should therefore prevent the formation of protein-associated strand breaks. Experiments designed according to this principle however provided strong evidence against a requirement for torsional tension.

The third question was whether the DNA in intercalator-treated cells does in fact undergo a change in helix winding number. This was tested by means of ethidium-titration nucleoid sedimentation. The results did indicate a change in helix winding number, but the change was in the wrong direction: a compensation for intercalator-induced torsional strain would have required a reduction in winding number, whereas an increase was observed.

Because of the hypothesized role of a topoisomerase-like enzyme, the effect of novobiocin, an inhibitor of some toposiomerases, was examined. Novobiocin was found to produce two separate and kinetically different inhibitions of the reversal of the intercalator-induced effects: (1) a slowing of the reversal of the protein-associated strand breaks, and (2) a more prolonged block of the recovery of the helix winding number.

Our new working hypothesis, consistent with all available data, is as follows: (1) intercalating agents can bind directly to a DNA-topoisomerase complex; (2) the resulting ternary complex tends to become trapped in an intermediate state with one or both DNA strands cleaved and the enzyme covalently linked to one terminus of each break; (3) the topoisomerase that is involved normally acts to reduce helix winding number, can generate double-strand breaks and is sensitive to novobiocin, suggesting that it is a DNA gyrase-like enzyme.

Studies are in progress to test and elucidate this mechanism. We have succeeded in extracting the intercalator-dependent enzyme activity from cell nuclei and in reconstituting the system using purified DNA. The enzyme activity has been partially purified by column chromatography. A quick assay for intercalator-dependent DNA-protein linking was devised to facilitate the fractionation. Our next objectives are to purify and characterize the enzyme and to determine its DNA sequence specificity and molecular mechanism.

Intercalator Effects on DNA: Biological Factors.

In the course of previous work to test the then prevalent idea that intercalator-induced DNA strand breaks are induced by a free radical mechanism, we had found an unexpected enhancement of this effect by dimethylsulfoxide. We have during the past year tested the possibility that this enhancement might have a common physical basis with the induction by dimethylsulfoxide of terminal differentiation. A structure-activity study however disclosed that other differentiation inducers of the polar-planar type failed to increase intercalator-induced strand breakage, and in fact tended to suppress it. The stimulation by dimethylsulfoxide is thus exceptional and remains unexplained.

The observation that the extent of intercalator-induced strand breakage can be affected by combination treatment of cells with other compounds suggested the test of drugs that are employed clinically in combination with intercalating agents. Arabinosylcytosine (Ara-C) was selected for this purpose. Pretreatment with Ara-C at concentrations that slowed cell proliferation, but allowed most cells to survive, was found to markedly increase the strand breakage induced by adriamycin or m-AMSA. A similar result was obtained with hydroxyurea and was correlated with an increase in the fraction of cells in S-phase. Accompanying the increase in intercalator-induced strand breakage, there were increases in intercalator-induced cell killing. These drug interactions may therefore have chemotherapeutic importance. The mechanism is also of interest because of the possibility that the enzyme that we hypothesize is blocked by intercalators -- i.e., an enzyme which actively unwinds the DNA helix -- may function in DNA replication. It is still necessary to assay for the enzyme activity as a function of the cell cycle and to study its possible role in DNA replication.

Intercalator-induced strand breakage was also enhanced by pretreating cells with 5-azacytidine. The enhancement occurred at the time when 5-azacytidine-induced DNA hypomethylation became evident. The significance of this observation is not clear, but it is interesting to note that, contrary to the case of Ara-C or hydroxyurea, the cells were not sensitized to m-AMSA-induced killing.

Histone Variant Synthesis Pattern in Relation to the Cell Cycle.

Members of the Chromosome Structure and Function Section had previously found that certain variant histone proteins show strikingly different relative synthesis rates compared to the S-phase pattern. Furthermore, different patterns were observed in quiescent (G_0) cells as opposed to G_1 or G_2-phase cells. These findings had provided a new biochemical window to the study of cell cycle controls, especially in regard to the G_0 state.

During the past year, studies were carried out on the functional nature of the histone synthesis that continues in the absence of DNA synthesis. Histones synthesized during G_1 and G_0 were found to become incorporated stably into nucleosomes. Preliminary studies however indicated that there may be some histone turnover in G_0 cells. The stimulation of DNA repair synthesis by UV did not increase the synthesis of G_0 or S-phase histone variants. Inhibition of DNA synthesis by hydroxyurea, or other S-phase specific inhbitors, suppressed the synthesis of basal histone variants (e.g. H2A.X and H2A.Z) to a much lesser degree than the inhibition of S-phase histone synthesis. Studies of a variety of DNA synthesis inhibitors is in progress in order to determine whether the remaining basal histone synthesis pattern is always the same.

The new biochemical criteria for G_0 as opposed to G_1-phase, based on histone variant synthesis pattern, allowed the question to be asked whether G_0 cells which are stimulated to enter the cell cycle will pass through a G_1 state prior to entering S-phase. Based upon the histone variant synthesis criterion, it was found that such cells do not pass through a G_1-like state before entering S.

Further studies will examine these regulation phenomena at the m-RNA and gene level. At the same time, the effects of drugs that inhibit various phases of the cell cycle will be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06112-13 LMPH

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October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanism of Action of DNA Reactive Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Leonard C. Erickson Sr. Investigator LMPH NCI

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John Strong, LCPH, NCI; John Groopman, Boston Univ., School of Public Health; David Ludlum, Albany, Medical College

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The mechanisms of action of the DNA reactive drugs: chloroethylnitrosoureas, AZQ, cis-Pt, and May&Baker 39565 have been investigated in human normal and tumor cell lines in vitro. The role of the repair of DNA chloroethyl monoadducts has been studied by inhibiting the repair protein with MNNG. Filter techniques have been developed to quantitate the number of potentially crosslinkable sites in DNA. These techniques should allow the study of adduct repair and its relationship to differential cytotoxicity of antitumor agents.

Project Descritpion:

Objectives:

(1) To apply filter elution techniques developed in this laboratory to the study of DNA damage produced by chloroethylnitrosoureas in human normal cells and tumor cell lines established in culture.

(2) To determine the ability of these cell lines to repair DNA chloroethyl monoadducts which are capable of producing DNA interstrand crosslinks.

(3) In cell lines resistant to chloroethylnitrosoureas, to determine whether the DNA repair system which prevents crosslinking can be inhibited.

(4) To determine the mechanism of action of the new antitumor agent M+B 39565, by studying the DNA damage produced in normal and SV-40 transformed human cells, and also in mouse L1210 leukemia cells.

(5) To determine the mechanism of action of AZQ in human tumor cell lines.

(6) To develop new applications of the alkaline elution technique, particularly in the study of crosslink formation from monoadducts, or potentially crosslinkable sites (PCS).

Methods:

(1) Alkaline elution filter techniques developed in this laboratory, to measure DNA strand breaks, DNA alkali-labile sites, DNA protein crosslinking, and DNA interstrand crosslinks.

(2) Human cell tissue culture systems including colony formation assays without soft agar, and a differential cytotoxicity assay which compares the survival of cell lines exposed to chemotherapeutic agents to untreated control cells which complete 3 or more population doublings.

(3) Isolation of chloroethylnitrosourea drug-DNA adducts by high performance liquid chromatography (HPLC).

(4) Identification of HPLC isolated adducts by mass spectrometer analysis.

Major Findings:

Inhibition of Chloroethyl DNA Monoadduct Repair by MNNG

Human cells are known to differ in their ability to repair methyl and ethyl lesions on the O^6- position of guanine. Repair proficient cells have been designated as Mer^+ (or Mex^+) and deficient cells Mer^- (or Mex^-). Normal human fibroblasts, and a variety of human tumor cell lines, are of the Mer^+ phenotype. We have previously shown that Mer^+ cells are capable of preventing DNA interstrand crosslink formation following treatment with chloroethylnitrosoureas. Our working hypothesis has been that in a Mer^+ cells a DNA repair system,

possibly the guanine O^6-alkyl transferase protein, has recognized O^6-chloroethyl monoadducts and removed these lesions before the second reaction of the adduct with the opposite DNA strand (a delayed reaction which takes 6-12 hr to occur). MNNG has been reported to be capable of inactivating the guanine O^6-alkyltransferase protein. Using non-toxic (2 μM, 1 hr) exposures of MNNG, followed by chloroethylnitrosourea (CNU) treatment for an additional hour, we were able to demonstrate CNU-induced DNA interstrand crosslinks in normal human fibroblasts (IMR-90) and human colon carcinoma cells (HT-29). Both cell lines are Mer^+, and no CNU-induced DNA interstrand crosslinking has been detected in these cells before. The MNNG pretreatment did not alter crosslinking levels in VA-13 cells (Mer^-) confirming that these cells have little or no chloroethyl repair capacity. Using colony forming assays we found that when we combined the nontoxic MNNG pretreatment with CNU treatments that produced little or no cell kill, synergistic cell killing was found to increase by 1-3 logs in the combination treatment. Thus we were able to show that by inhibiting the DNA repair system that protects cells against crosslink formation, we could produce DNA crosslinks, and greatly increase cell kill.

Comparison of Chloroethylnitrosoureas Having Low and High Carbamoylating Activity for Cytotoxicity in Normal Human Cells, and for Inhibition of Radiation Induced DNA Strand Break Rejoining

Previous work from our laboratory had suggested that two chloroethylnitrosoureas with high carbamoylating activity (BCNU, CCNU) were more toxic to normal human cells than the non-carbamoylating nitrosourea chlorozotocin. Since one goal in increasing the therapeutic index in chemotherapy is the protection of critical normal tissue we have studied the cytotoxicity to normal human fibroblasts (IMR-90) of 6 chloroethylnitrosoureas which differ in carbamoylating activity (CNU, BCNU, CCNU, PCNU, cis-2-OH-CCNU, and trans-4-OH-CCNU). As a test of the ability of the carbamoylating moiety to inhibit an enzymatic process, we also monitored DNA strand rejoining in cell cultures treated with the drugs and irradiated with 1000 R x-irradiation. Using colony formation assays the cytotoxicities of the drugs were found to fall into two groups: CNU, PCNU and cis-2-OH-CCNU produced only modest cell killing, whereas BCNU, CCNU and trans-4-OH-CCNU produced significantly higher cell killing. When the ability of the drugs to inhibit x-ray repair was tested following 1000 R irradiation, the 3 nitrosoureas which produced the higher cell kill inhibited the strand rejoining over a period of several hours following irradiation. In the group of drugs producing the lower cell kill (CNU, PCNU, cis-2-OH-CCNU) little or no inhibition of DNA strand rejoining was observed. Collectively these data suggest that with the two nitrosoureas used most frequently in the clinic (BCNU, CCNU) survival of critical normal tissue may be reduced by the ability of these drugs to inhibit enzymatic processes in cells that should normally survive well because they are proficient in DNA monoadduct repair (see previous section). Studies are currently underway to select a chloroethylnitrosourea with low carbamoylating activity that might be tested in clinical trials for increased therapeutic index.

Studies on the Mechanism of Action of AZQ

AZQ, an aziridinylbenzoquinone derivative, was found to have DNA strand breaking and interstrand crosslinking activities in mammalian cells exposed to this drug.

We have studied the production of DNA single strand breaks (SSB) and interstrand crosslinks (ISC) by AZQ in SV-40 virus transformed human fibroblasts (VA-13) and isolated VA-13 nuclei using alkaline elution technique. Both lesions were produced in intact cells as well as in isolated nuclei. SSB formation in isolated nuclei was dependent upon the presence of NADPH, and was inhibited by superoxide dismutase. ISC formation also was NADPH dependent but did occur in the presence of superoxide dismutase. Preincubation of AZQ with NADPH prior to nuclei treatment enhanced the crosslinking activity of the drug. Very high crosslink frequencies and rapid formation of this lesion were found when nuclei were treated with AZQ in the presence of a strong reducing agent, $NaBH_4$. An alkylating test with 4-(p-nitrobenzyl)pyridine showed that the reduced form of AZQ exhibited markedly higher alkylating activity than did the quinone form. The present results suggest that DNA breakage is caused by free radicals which are generated in the reaction of the AZQ semiquinone with oxygen. The reduced form of the drug seems to be responsible for DNA interstrand crosslinking.

Studies on the Mechanisms of Action of May&Baker 39565 (NSC 353451)

L1210 murine leukemia cells were treated in vitro with the novel antineoplastic agent M&B 39565 (8-carbamoyl-3-(2-chloroethyl)imidazo|5,1-d|1,2,3,5|tetrazin-4(3H)-one and its interaction with cellular DNA was assessed by the technique of alkaline elution. DNA interstrand crosslink and DNA-protein crosslink formation was quantified with regard to drug concentration and length of incubation time post a 2 hour incubation period with drug. Cytotoxicity as measured by colony formation assays, and DNA damage caused by M&B 39565, were compared with that caused by a breakdown product of M&B 39565, MCTIC (5-(3-(2-chloroethyl)triazen-1-yl)imidazo-4-carboxamide) and also with CNU (1-(2-chloroethyl)-1-nitrosourea). Both MCTIC and CNU decompose to yield a 2-chloroethyldiazo species which is capable of alkylating DNA. At equimolar concentrations all three drugs possessed similar in vitro cytotoxicities and at equitoxic concentrations produced similar levels of DNA interstrand crosslinking.

Normal (IMR-90) and SV-40 transformed (VA-13) human embryo cells were treated with M&B 39565 and the effects on cell viability and cellular DNA integrity were studied. M&B 93565 and MCTIC were 5-6 fold more toxic to VA-13 cells than to IMR-90 cells for drug concentrations which produced a 2 log cell kill, as measured by colony forming assays. Using alkaline elution analysis VA-13 cells exhibited concentration dependent DNA-interstrand crosslink formation. However, in IMR-90 cells little or no interstrand crosslink formation was detected. A linear correlation between DNA interstrand crosslink formation and log cell kill was observed in VA-13 cells, but not in IMR-90 cells. DNA protein crosslink formation was found to be comparable in both cell lines for each drug, suggesting that drug penetration and intracellular drug reactivity was similar. Initial chemical decomposition studies suggest that both M&B 39565 and MCTIC may produce a chloroethyldiazohydroxide species. This species has been implicated in the formation of chloroethyl-DNA adducts which convert to DNA interstrand crosslinks in mammalian cells treated with chloroethylnitrosoureas. These studies suggest that M&B 39565 is cytotoxic as a consequence of DNA interstrand crosslink formation and although similar in some respects to the nitrosoureas, such as CNU, may present distinct advantages over the nitrosoureas currently used in the clinic, such as BCNU and CCNU, because of its lack of carbamoylating

activity. (See Section on Comparison of Chloroethylnitrosoureas.....).

Development of Filter Assays for the Measurement of DNA Monoadduct Conversion to Interstrand Crosslinks (Potentially Crosslinkable Sites (PCS))

Chloroethylnitrosoureas are known to be capable of producing DNA interstrand crosslinks in isolated DNA reacted with drug in saline. The formation of the crosslink was in a delayed reaction after drug removal and took place over a 6-18 hour period. We reasoned that monoadducts were produced in the DNA of cells treated with drug, and the DNA subsequently isolated by lysing the cells on a membrane filter, might be able to complete crosslink formation if the filters were incubated in a buffered solution at 37°. If this procedure worked we would be able to assay cells for the maximum level of crosslinking, since all cellular DNA repair processes would be eliminated. The assay for potentially crosslinkable sites (PCS) was carried out in VA-13 cells (Mer^-, see section 1) treated with chloroethylnitrosourea (CNU). It was found that after drug treatment for 1 hr, followed by lysis of the cells on 0.8 μ polycarbonate filters, DNA interstrand crosslinks would accumulate over a 12 hr period reaching a plateau at between 6 and 9 hours. The plateau was found to increase in a dose dependent manner following increasing drug concentrations. Further experiments showed that another DNA crosslinking agent cis-diamminodichloroplatinum(II) (cis-Pt) also produced dose dependent crosslinking levels over a similar time period. Using this methodology we should be able to determine: (1) how rapidly are CNU-monoadducts removed in Mer^+ cells? (2) How much cis-Pt mono adduct repair occurs in different cell lines? (3) What are the kinetics of monoadduct repair, and conversion of monoadducts to crosslinks with other cross linking agents such as L-PAM, nitrogen mustard, and cytoxan?

High Performance Liquid Chromatography (HPLC) of DNA Adducts Produced in DNA Reacted with Drug In Vitro, and in DNA Isolated from Cells Reacted with Drug

In collaboration with Dr. John Groopman of Boston University School of Public Health, and Dr. David Ludlum of Albany Medical College, we have studied the adducts formed in L1210 DNA reacted with drug in vitro, and in DNA isolated from L1210 cells following drug treatment. Using an acetonitrile/water gradient HPLC scheme developed by Dr. Groopman we have been able to isolate at least (8) eight different DNA adducts from DNA reacted with chlorozotocin, or cells reacted with ^{14}C-labeled PCNU. Preparative HPLC was used to produce and purify sufficient quantities of material to identify some of the adducts by mass spectrometry. These analyses were performed by Dr. John Strong, LCPH, NCI. Thus far 7-chloroethylguanine, 7-hydroxyethyl-guanine, and O^6-hydroxyethyl-guanine have been identified. In addition to these adducts Dr. David Ludlum has identified a di-guanyl-ethane crosslinked adduct, and possibly a guanine-cytosine ethane crosslinked base pair from cells exposed to ^{14}C-CCNU.

Proposed Course:

1) Study the repair of the class of chloroethylnitrosourea-induced DNA monoadducts that are capable of forming interstrand crosslinks, both in human cells and in cell-free extracts. Are the kinetics consistent with the speed that is required for this repair to prevent ISC formation? Is this repair mediated by

the same enzyme activity that is responsible for methylation repair?

2) Compare the relative quantities of DNA adducts produced by different presumed chloroethylating agents, both in purified DNA and in cells, using HPLC methodology. The eventual objective is to narrow the range of DNA lesions produced by nitrosourea-related drugs to include only those lesions that contribute to anti-tumor activity.

3) Determine DNA site-specificity for interstrand crosslink formation by bifunctional alkylating agents. Are there hot spots for the formation of ISCs, depending on DNA sequence or conformation? Are different sites selected depending on chemical structure of the alkylating agent? Are particular sites favored in DNA when it is packaged in chromatin? The objective is to find means of attacking selectively certain DNA regions in the cell.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06140-07 LMPH

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Protein Interactions in Chromosomes; Cell Cycle and Cell Proliferation Controls

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

William Bonner	Sr. Investigator	LMPH NCI
Roy S. Wu	Cancer Expert	LMPH NCI

COOPERATING UNITS *(if any)*
Department of Biological Chemistry, School of Medicine, Univ. of California, Davis; Department of Biochemistry, GWU Medical School and the Department of Biology, Georgetown University, Biotech Res. Labs. Inc.

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION
Chromosome Structure and Function

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

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3	2	1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Using methodology developed in our group over the last several years to resolve and characterize histone variants, we have been analyzing the patterns of histone synthesis during different cell behavioral states. Histones were found to be synthesized not only during S-phase, but also during G_1 and the quiescent state (also termed extended G_1 or G_0). The qualitative pattern of histone synthesis differs between S-phase, G_1, and quiescent cells, a finding which shows that the synthesis in G_1 or quiescent cells is not due to contamination by S-phase cells. The histone synthesis in both G_1 and quiescent is not linked to DNA synthesis. Histones synthesized in quiescent cells are stable and are rapidly incorporated into nucleosomes. The results suggest that the quiescent state is not an extended G_1 but a discrete state or cycle. Studies are in progress with histone mRNA's and genes, in order to elucidate this phenomenon at the gene level.

Project Description

Introduction:

When cells cease division and become quiescent, they continue to synthesize histones at a reduced but significant rate. The pattern of variant synthesis in quiescent cells differs from that in S-phase; therefore, this synthesis cannot be attributed to the presence of S-phase cells in the culture (Wu, Tsai, and Bonner, Cell 31: 367-374, 1982).

In CHO cells where the pattern of histone synthesis has been examined during the cell cycle, reduced but significant synthesis has been found in both G_2 and G_1 (Wu and Bonner, Cell 27: 321-330, 1981). This basal pattern differs from both the S-phase and the quiescent patterns. These results strongly suggest that the quiescent state is not merely an extended G_1, but is a discrete state or cycle.

Specifically, the synthesis of histone 3 variants .1 and .2 is turned off in quiescent cells. Cell lines IMR-90, 3T3, and CHO as well as normal human lymphocytes all show this change between cycling and quiescent states, indicating that this is a general phenomenon. The histone synthesis in quiescent cells is unaffected by treatment with inhibitors of DNA synthesis such as hydroxyurea, indicating that this synthesis is not linked to DNA synthesis.

HISTONE VARIANTS SYNTHESIZED IN VARIOUS CELL STATES

S-phase	Quiescent	Basal (G_1, G_2)
H4	H4	H4
H2B	H2B	H2B
H2A.1	H2A.1	---
H2A.2	H2A.2	---
H2A.X	H2A.X	H2A.X
H2A.Z	H2A.Z	H2A.Z
H3.1	---	---
H3.2	---	---
H3.3	H3.3	H3.3

Objectives:

A. To study the function of quiescent and basal histone synthesis. This includes investigating the fate of the histones synthesized in quiescent and G_1 cells and the possible involvement of these histones in such processes as transcription, cell cycle timing, maintenance of chromatin, and DNA repair.

B. To use the patterns of histone synthesis to study the relationship of different cell behavioral states, particularly those other than S-phase.

C. To study the differential controls on histone synthesis at the mRNA and gene levels in order to help elucidate the factors that control cell cycling and proliferation behavior.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional eletrophoresis. (Methods developed in this laboratory).

(2) Peptide analyses on acrylamide gels to determine the relationship of proteins to each other. (Method developed in this laboratory).

(3) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(4) Maintenance of cells in viable non dividing states due to nutrient starvation, serum deprivation, or differentiation.

(5) Isolation and analysis of mRNA from different parts of the cell cycle or from quiescent cells.

(6) Cell free translation of mRNA in wheat germs extracts.

(7) Preparations to screen for genes of histone variants including hybrid select and hybrid arrested translation, and various kinds of blotting.

Specific Projects:

A. Function of Histones Synthesized in Non-S-Phase Cells

1. Fate

This study includes the fate of these newly synthesized histones. Basal histones synthesized in G_1 are stable and present several generations later; they are found in mononucleosomes within 0.5 hr. Histones synthesized in quiescent cells are also stable and are rapidly incorporated into mononucleosomes.

2. Turnover

The results just discussed raise the possibility of histone turnover. Preliminary experiments comparing histone variant content of whole tissues with that of cells grown *in vitro* suggest that there may be histone turnover in quiescent cells. Experiments to document this process are in progress.

3. DNA Repair and Chromatin Maintenance

Using normal human lymphocytes, we have studied the effect of stimulating DNA repair by UV on quiescent histone synthesis. Stimulating DNA repair synthesis does not stimulate the synthesis of either quiescent or S-phase histone variants. Because there is already significant histone synthesis in these cells,

interpretation of these results will depend on the results of turnover studies.

4. Linkage of Quiescent, and S-Phase Histone Synthesis to DNA Synthesis

Many studies have shown that inhibition of DNA synthesis immediately leads to a similar inhibition of histone synthesis even though total protein synthesis is not significantly inhibited. Our studies with hydroxyurea, a classical inhibitor of DNA synthesis, show that basal and S-phase histone synthesis are inhibited to different extents when DNA synthesis is inhibited. While hydroxyurea inhibited the synthesis of S-phase specific variants to less than 10% of their control levels the basal variants still maintained 26% to 34% of their control levels. The differential inhibition of basal and S-phase histone synthesis could be due to a differential linkage of these two types of synthesis to DNA sythesis or to another effect of the hydroxyurea. Comparing several types of DNA synthesis inhibitors and antitumor antimetabolites could enable us to distinguish between these possibilities. We are presently doing such a study with Drs. Leonard Erickson and Eric Sariban.

Quiescent histone synthesis is also resistant to inhibition of DNA synthesis by HU; this evidence supports other evidence including the DNA repair studies that quiescent histone synthesis is not linked to DNA synthesis.

B. Use of Histone Synthesis Patterns to Study the Relationship of Various States of Cell Behavior

1. Quiescence and G_1

As discussed in the Introduction, quiescent histone synthesis and basal (G_1, G_2) histones synthesis do not exhibit the same variant pattern (Cell 31: 367-374, 1982.

This finding indicates that quiescent cells are not simply arrested at some point in G_1, but are in a discrete state or cycle. Using cells in appropriate states, we are investigating when the decision to enter or exit quiescence is made. Results of histone variant synthesis patterns indicate that quiescent cells do not pass through G_1 before entering S, but enter S from the quiescent state after an appropriate lag.

2. Other States

Studies are being initiated using defined media to study factors which influence cells to cease or initiate proliferation. This is an extremely broad area, but presently our objectives are limited to studying whether several kinds of quiescent states may exist. Quiescent states induced by nutrient deprivation, serum deprivation, or differentiation all seem to exhibit the same pattern of quiescent histone synthesis.

C. Cell Proliferation and Cell Cycle Controls

1. mRNA Studies

mRNA from cells in various phases of the cell cycle and states of cell prolife-

ration have been isolated and translated in cell free lysates from wheat germ. Experiments are being initiated to study the linkage of various kinds of histone synthesis to DNA synthesis at the mRNA level. Since inhibition of DNA synthesis shuts down certain types of histone synthesis and not others, differences in mRNA or polysome structure are possible explanations. Separation of mRNA into Poly A^+ or poly A^- fractions has shown that separation of poly A content of mRNA is probably not related to this phenomenon.

D. Histone Protein Studies

1. Histone Variants in Evolution

This spring we were awarded a NATO Collaborative Fellowship to study Histone Variants in Evolution with Dr. H. Pataryas, Professor of Biology at the University of Athens, Greece. Dr. Pataryas visited this laboratory in the summer 1981, at which time he proved the feasibility of this approach. The fellowship will be used to set up the study in his laboratory in Athens.

2. Acellular Slime Mold, Physarum

We are doing a collaborative study with Professor Morton Bradbury, Head of the Department of Biological Chemistry, School of Medicine in Davis, California, on the acellular slime mold, Physarum. The preliminary results indicate that Physarum does contain a histone which migrates with or at least very close to mammalian H2A.Z. We hope to be able to compare the fingerprints of these proteins this summer.

E. Histone Modification

Histones are modified by acetylation, phosphorylation, methylation, poly-ADP ribosylation, and ubiquitination of various amino acid side chains. Our purpose here is to develop more rigorous procedures for separating and quantitating the various forms in the complex mixtures of modified histones normally present in living cells, and to study whether some insight can be gained as to the functional roles of these modifications. It should be noted that up to now it has only rarely been possible to quantitate various types of histone modifications.

Just before he left, Dr. Pantazis discovered that altering the ion content of the growth media can lead to greatly increased phosphorylation of H2A. This promises to be a useful tool for studying not only how phosphorylation alters chromatin structure and function, but also how the chromatin may react to environmental influences. Lowering the pH of the growth media has a similar but not identical effect.

F. H4 Expression in an SV40-Mouse H4 Gene Hybrid

Dr. Ajit Kumar of the George Washington University Medical School and Dr. Dean Hamer of the Laboratory of Biochemistry, DCBD have constructed a hybrid between SV40 DNA and mouse H4 gene. They have shown that the H4 region is transcribed using the late SV40 promoter. The question we are concerned with is whether or

not the resulting mRNA is translated to give mouse histone 4. Since mouse H4 and monkey H4 (the host for SV40) are identical proteins, the answer must be a quantitative one rather than a qualitative one. Therefore, we compared the ratios of the four nucleosomal histones in Green monkey kidney cells infected with wild-type SV40, a hybrid between SV40 and globin DNA, and the SV40-H4 hybrid. When given a 10 minute pulse of ^{14}C-arginine, the first two infections showed equimolar synthesis of the four nucleosomal histones. The third infection with the SV40-H4 hybrid showed equimolar synthesis of the H3, H2A and H2B with a 3 to 4 fold excess synthesis of H4. These results are a strong indication that H4 is translated from the mRNA transcribed from the SV40-H4 genome.

Significance to Biomedical Research and Program of the Institute:

Cancer at one level is the inappropriate multiplication of cells. Our findings during the last year have suggested that analysis of histone variant synthesis and the histone variant genes may yield some insight into the relationship of different cell states including neoplastic states.

Proposed Course:

1. To study the fate and function of histones synthesized in quiescent, G_1, and G_2 cells.

2. To gain insight into the relationship of different cell states through their pattern of histone synthesis.

3. To study the differential controls on histone synthesis at the mRNA and gene levels in order to help elucidate the factors that control cell cycling and proliferation behavior in normal and neoplastic cells.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06150-02 LMPH

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cell Biology and Biochemistry of DNA Intercalating Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Leonard A. Zwelling Senior Investigator LMPH NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

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SECTION

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A uniform effect of DNA intercalating agents is the production of protein-associated DNA scission in mammalian cells following their exposure to these drugs. The precise biochemistry of this effect and its relation to drug effects on a host of biological events is unknown. We have shown that the production of this effect has several characteristics of an enzymatic process including saturability, temperature-dependence and reversibility. In the past year we have enhanced our understanding of the relationship of this DNA scission to drug-induced cytotoxicity, to the cell cycle phase at the time of intercalator treatment, to hormonal effects on cellular chromatin and to specific alterations in 2- and 3-dimensional chromatin structure. In addition, partial purification of the enzymatic activity producing the DNA scission has been accomplished and a totally in vitro system for reconstituting and thus characterizing this novel effect now exists. Finally, a clinical trial for the treatment of acute nonlymphocytic leukemia has been initiated which utilizes the principles discovered in our laboratory as well as the results of a completed phase I-II trial of continuous infusion m-AMSA.

Project Description:

(1) Study the biochemistry of the protein-associated DNA scission produced by DNA intercalating agents.

(2) Quantify this effect and its relationship to other measurable cell functions including colony-forming ability (in both synchronized and asynchronous cell populations), DNA synthesis, growth rate, transcription, differentiation, mutation, and chromosomal integrity.

(3) To combine the biochemical and cell biological information to identify model systems in which intercalating agents could act as specific probes for variations in chromatin structure which are concomitants of specific intracellular events.

(4) To incorporate these biochemical and pharmacologic principles into rational clinical trials for the improved treatment of human malignancies displaying sensitivity to intercalating agents.

Methods Employed:

(1) Cell culture of various rodent, primate and human cell lines -- treatment with hormonal and other pharmacologic agents *in vitro*; radioactive labeling of macromolecules.

(2) Isolation of cell nuclei

(3) Nucleoid sedimentation

(4) X-irradiation of cells and subcellular components

(5) DNA break and crosslink assays on membrane filters.

(6) Soft agar colony formation of rodent cells, and colony-forming assays of human breast cancer cells.

(7) Radioactive drug uptake and egress.

(8) SV-40 virus infection of primate cells and subsequent isolation therefrom.

(9) HPLC analysis of DNA methylation

(10) Flow microfluorimetry

(11) Sephacryl-gel chromatography

(12) DNA cellulose chromatography

(13) SDS-polyacrylamide gel electrophoresis

(14) Agarose gel electrophoresis of DNA.

Major Findings:

1. Biochemistry

(a) Intercalator-induced DNA binding activity in a nuclear extract.

Previous work by Filipski et al. (in press) had demonstrated that the intercalator dependent DNA breaking activity present in isolated L1210 murine leukemia cell nuclei was extractable with 0.35 M NaCl. An extract so prepared could be used to reconstitute detectable intercalator-dependent DNA breaking activity in salt-depleted chromatin. The biochemical characterization of this system was not performed. However, Filipski did note that the activity was localized to a high molecular weight peak following gel filtration which did not correlate with the peak of topoisomerase I activity.

We have continued this work by streamlining the assay for enzyme activity from 2 days (overnight filter elution) to 15 min (rapid filter binding by intercalator-induced DNA-protein crosslinks in the presence of the enzyme extract). This has allowed more accurate and rapid identification of the major peak of activity following Sephacryl S-400 chromatography. Additionally, our next purification step was rapidly identified as DNA cellulose onto which enzyme activity can be rapidly bound and released by adjustments in the salt concentration of eluting buffers. This purification continues.

(b) The use of DNA of defined sequence-in vitro.

All the prior work assaying the intercalator-dependent DNA effects of the salt-extracted (0.35 M) nuclear extract was performed with salt-extracted L1210 cell DNA as a substrate. We have reproduced this intercalator-dependent, temperature-sensitive DNA-protein crosslinking activity of the nuclear extract using protein-free SV-40 viral DNA as a substrate. The DNA binding site of the extract protein(s) can be quantitatively and qualitatively studied as the entire SV-40 sequence is defined.

(c) The use of DNA of defined sequence-in vivo.

In addition to studying the intercalator-induced DNA-protein crosslinking and breaking activity using isolated SV-40 DNA in vitro, we have been able to produce both DNA breaks with intercalators in the SV-40 DNA located within "minichromosomes" in the nuclei of CV-1 monkey kidney cells. That is, cells infected with SV-40 are treated with the intercalator m-AMSA and the viral DNA can then be extracted and characterized. Thus intercalator effects on an intracellular DNA of known sequence and structure can be studied in a system in which the cellular pharmacologic parameters are identical to those under which whole cellular DNA effects are seen. The temperature sensitivity and reversibility characteristics of the intercalator-induced, protein-associated DNA scission in whole cell and isolated nuclei systems is also characteristic of the intercalator effects on SV-40 DNA in vivo.

(d) Nucleoid sedimentation studies of intercalator-induced alterations of the cellular DNA.

Nucleoids are DNA-protein structures produced by lysing whole cells in non-ionic detergent and sedimenting the cell lysates in neutral 1.9 M NaCl-sucrose (pH 8). Nucleoid sedimentation is a very sensitive measure of various DNA effects (including scission) produced by DNA-reactive agents within the cells from which the nucleoids were isolated. This technique creates a sedimenting structure of DNA and small amounts of protein. Histones and most non-histone chromosomal proteins are removed by the detergent and high salt treatments. The small amount of residual protein is thought to retain the DNA in loops or domains which may actually exist in vivo.

Previously we had shown that nucleoids from m-AMSA-treated cells sedimented identically to nucleoids from untreated cells. This was surprising given the large amount of DNA scission detected within the DNA from m-AMSA-treated cells using filter elution or alkaline sedimentation technology. We reasoned that as the DNA scission detected in alkaline elution assays was undetectable without enzymatic proteolysis of cell lysates prior to elution, a similar step could be required to render underlying m-AMSA-induced scission detectable in nucleoid sedimentation assays. Such was indeed the case. Limited proteinase-K treatment exposed the underlying, intercalator-induced scission without altering the sedimentation of nucleoids from control cells. Several conclusions could thus be drawn:

(1) Intercalator-induced, DNA-protein crosslinking was present in sedimenting nucleoids.

(2) These protein crosslinks must span the underlying breaks and prevent swivelling around these breaks as any such swivelling would have led to a relaxed, decompacted nucleoid which would have sedimented slowly in assays without proteinase.

(3) Our previous model in which protein crosslinks were covalently created at DNA scission sites bound to one terminus of the break may not wholly explain the structure of the DNA break site. The proteins seem to bridge the breaks with the protein-DNA bonds on either side of the break having different characters. One bond is likely to be covalent and requires enzymatic digestion to break. The other is non-covalent, dissociable by detergents or alkali and thus seen intact only in the nucleoid sedimentation assay performed at pH 8.

Nucleoid sedimentation can also be adapted to give further information about the cellular DNA from which it was isolated. By including various concentrations of the intercalator eithdium bromide within the salt gradients through which the nucleoids sediment, various states of DNA compaction can be produced. That is ethidium intercalation will unwind the DNA until a completely unwound structure is obtained. Further, higher concentrations of ethidium will then rewind the DNA in the opposite direction (providing no strand breaks are present).

Within intact double-stranded DNA, three parameters define its three-dimensional structure. Linking number (L_k) is the number of times one strand winds about the other. L_k can be altered within a given DNA only through DNA breakage. Twist (T_w) is the number of base pairs per DNA molecule divided by the number of base pairs per complete helical turn. It reflects the angle between adjacent

base pairs and is decreased by intercalation. Writhe (W_r) is a measure of supercoiling, defined by $W_r = L_k - T_w$. W_r (or supercoiling) is negative within the living cell; that is T_w exceeds L_k.

Once a nucleoid is isolated, however, only L_k is a topological invariant from those parameters extant within the living cell prior to isolation. However, when an ethidium titration is performed, the concentration at which nucleoid sedimentation distance is minimal represents the point of complete nucleoid unwinding (decompaction). At this point $W_r = 0$ and $T_w = L_k$. Performing ethidium titrations on nucleoids from m-AMSA-treated vs. control cells revealed an alteration of the ethidium titration profile in nucleoids from drug-treated cells. It took a lower concentration of ethidium to completely unwind the nucleoids from m-AMSA treated cells suggesting the drug treatment had resulted in an altered L_k within the cells. This suggests that either the absence of swivelling at the protein-bridged break sites within nucleoids may not mimic the events within cells, or that DNA breakage and swivelling occurred within the cells at locales other than at the nucleoid-detectable scission sites which themselves are not detectable within nucleoids.

Regardless, the findings gleaned within nucleoids of m-AMSA-induced, protein-bridged DNA scission and alteration in cellular DNA L_k substantiates our hypothesis that topoisomerase action or its inhibition are somehow related to the effects of intercalators on cellular DNA. In fact, novobiocin, an inhibitor of type II topoisomerase, can interfer with the reversibility of some of these m-AMSA effects.

(e) Nuclear scaffold-alterations by intercalators.

Preliminary work has begun examining nuclear scaffold proteins using the isolation technique of Lebkowski and Laemmli (JMB 156: 325, 1982) for alterations produced by m-AMSA. Changes in some of these proteins have been identified in autoradiographs of SDS-polyacrylamide gels, but work on this aspect of m-AMSA's effects on nuclear structures is still in its nascent stages.

(f) The use of isolated cell nuclei to characterize intercalator-induced DNA scission.

We previously showed that reversible, intercalator-induced DNA scission and protein-crosslinking could be produced in isolated cell nuclei. These studies have been extended to characterize temperature, pH, and divalent cation optima for the DNA single- and double-strand scission produced by m-AMSA, 5-iminodaunorubicin and ellipticine. A new method of double-strand break quantification had to be developed for this purpose.

Perhaps most-importantly, we were able to show that prior DNA scission produced by x-radiation was without effect on the DNA scission produced by subsequent intercalator treatment. If intercalator-induced scission depended upon the production or alteration of torsional tension within cellular DNA, as we had previously believed, x-ray-induced breaks should release this tension and thus render the DNA target an inferior one for the detection of intercalator-induced scission. This did not occur. We believe torsional tension or alterations

therein is not related to intercalator-induced scission.

(2) Cellular Biology

(a) The effects of pharmacologic manipulation of the distribition of cells within cell cycle phases upon intercalator-induced DNA scission and cytotoxicity.

Drug combinations are commonly employed for the treatment of human malignancy. Pre-clinical pharmacology is rarely performed prior to the introduction of such therapy. Regimens employed in the treatment of acute nonlymphoblastic leukemia commonly include cytosine arabinoside (Ara-C) and an anthracycline intercalator (e.g. daunorubicin). Mechanisms explaning the efficacy of this combination are unknown.

The concept that rapidly dividing populations of cells progress through phases of a cell cycle is well accepted. These phases are defined by the DNA content of the cells. Post-mitotically cells have a DNA content equal to a complete genome (2n). This is G_1. S-phase is characterized by an increasing DNA content. G_2 is the immediate pre-mitotic period when DNA content is 4n. Cells then divide. The use of flow microfluorimetry (FMF) has allowed a large cell population to be characterized as having a certain percentage of its cells within the different phases of the cell cycle.

DNA content, however, is unlikely to be sufficient to totally characterize dividing cell populations. Yet, further biochemical characterization of the phases of the cell cycle are few. We were interested in the susceptibility of cell populations with different FMF profiles (DNA histograms) to the DNA breaking and cytotoxic actions of intercalating agents. We felt this was of interest from the viewpoint of potential clinical applications as well as from a more basic viewpoint. As DNA must alter its structure (unwind) to replicate and as topoisomerase action may be required to facilitate this unwinding, we wondered whether cell populations enriched in S-phase through pharmacologic manipulation might differ from unsynchronized populations in their susceptibility to protein-associated DNA scission, a biochemical event putatively related to topoisomerase action.

L1210 mouse leukemia cells were treated for various periods of time with either Ara-C or hydroxyurea (HU), another commonly employed antileukemic agent. Ara-C and HU doses were chosen so as to inhibit growth rate by only 50% and reduce colony-formation to only 80% of control values. Despite the minimal effects produced by Ara-C or HU themeselves, they greatly potentiated the susceptibility of cells to both the DNA breaking and cytotoxic actions of the intercalators m-AMSA and adriamycin. The enhancement of DNA and cytotoxic effects exhibited similar time-dependent and dose-dependent relationships to Ara-C or HU treatments. Further, the enhancement required at least 6 hr of Ara-C or HU treatment prior to its detectability. It was maximum at 18 hr of Ara-C or HU exposure and rapidly disappeared once Ara-C or HU were removed. This time course was not consitent with that of the Ara-C or HU inhibition of DNA synthesis which occurred much more rapidly. It was, however, consistent with the time to recruit cells into S-phase. The DNA content of S-phase was not the critical parameter, however, as 24 hr following Ara-C or HU removal a wave of cells

passed through S, but this wave did not display the enhanced susceptibility to m-AMSA seen in a cell population of comparable DNA content immediately following Ara-C or HU treatment. Biochemical events within cells pharmacologically recruited into S-phase appear to be critical to the enhanced action of intercalating agents within these cell populations.

(b) Pharmacologic alterations in DNA methylation and their relationship to m-AMSA-induced DNA scission and cytotoxicity.

Among the structural characteristics of DNA from cells with actively transcribing chromatin is a decrease in the amount of 5-methyl cytosine present when compared to DNA from non-transcribing cells. The hypomethylated state can be induced in cells by 5-azacytidine and this drug has been associated with the production of cellular differentiation at doses producing decreased methylation. The mechanism by which altered DNA methylation triggers gene expression is not known, but could result from local alterations in the 3-dimensional structure around sites of altered methylation which act as switches for gene expression. Recent findings that conversion of DNA from the B to the Z state can also depend on cytosine methylation could eventually explain such switching. We have demonstrated that the DNA from 5-azacytidine treated cells will display more m-AMSA-induced, protein-associated DNA strand breaks than untreated cells. This effect is most clearly demonstrable 24 hr following 5-azacytidine treatment as would be expected if cell division and DNA synthesis on a hypomethylated template were required prior to the cellular DNA acquiring its new more readily scissioned configuration.

We have confirmed that the 5-azacytidine dose we employed does block methylation using high pressure liquid chromatographic analysis of methyl-cytosine residues from cellular DNA. However, both Ara-C and HU also inhibit DNA methylation. Several critical differences exist, however, between the results with Ara-C and HU and those with 5-azacytidine. Although 5-azacytidine did enhance intercalator induce scission, it did so only following a 24 hr incubation in drug-free medium. Ara-C and HU enhanced DNA's susceptibility to breakage by m-AMSA immediately following their removal. 5-azacytidine did not potentiate m-AMSA's cytotoxicity (vide supra).

It would appear then that pharmacologic manipulation of cell populations can serve to dissociate drug effects potentiating m-AMSA's cytocidal effects from effects potentiating its DNA breaking effects. The recruitment of cells into S-phase by Ara-C and HU clearly create a biochemical alteration distinct from that created by inhibition of cytosine methylation alone.

(c) Hormonal treatment of hormonally-responsive human breast cancer cells and its relationship to intercalator-induced DNA scission.

We have previously reported on enhancement of m-AMSA and 5-iminodaunorubicin-induced DNA scission in hormonally-responsive human breast cancer cells (MCF-7) following 10-24 hr of exposure to estradiol (maximum effect at 10^{-9}-10^{-8} M E_2). We are now utilizing these cells to discern whether this effect is secondary to alterations in chromatin structure which accompany an enhancement in transcriptionally-active chromatin or the effect is secondary to other hormonally-induced effects such as alterations in growth rate or cell cycle phase distri-

bution. The finding that MCF-7 cells at higher cell densities grow more rapidly than cells at low densities and the finding that this appears to be secondary to a soluble factor produced by growing MCF-7 cells has allowed us to begin experiments which can dissect estrogen effects on growth from those at the chromatin level.

(d) The relationship between chemical stimulation of differentiation and intercalator-induced DNA scission.

Previously we had shown that dimethyl sulfoxide (DMSO) could produce in cells an alteration in DNA structure which accompanied a potentiation of m-AMSA's ability to produce DNA scission. We have extended this work to examine other promoters of differentiation to see if they, like DMSO, altered the susceptibility of cellular DNA to DNA scission. Neither tetramethylthiourea nor N-methylnicotinamide enhanced m-AMSA's DNA scission capabilities. In fact, higher concentrations of these agents supressed break formation. A simple chemical relationship between compounds capable of producing differentiation in some human leukemic cell lines and the enhancement of intercalator-induced DNA scission in L1210 cells apparently does not exist.

(3) Clinical Trials

We have previously demonstrated our ability to continuously infuse m-AMSA intravenously for 3-4 days with minimal toxicity. Efficacy would be expected only in diseases responsive to m-AMSA given in the more standard bolus fashion. The primary disease entity in which m-AMSA has displayed its activity is adult acute leukemia, a disease not routinely studied at the NCI.

However, the occurrence of a particularly resistant variant of this disease following treatment for other neoplastic conditions means a cohort of potential leukemic patients already exist within the NCI clinics. No protocol study existed for their treatment. We have combined continuous m-AMSA infusion as induction therapy followed immediately by high dose Ara-C and daunorubicin (MB-169). This will allow us to determine the remission induction rate of single-agent m-AMSA in acute leukemia while denying no patient exposure to more conventional forms of therapy. Additionally cytogenetic analysis, biochemical and pharmacologic studies will be performed on the malignant cells from these patients in an attempt to determine mechanisms of drug resistance, the presence of onc gene RNA, the presence of DNA within these cells which maintain the malignant phenotype, as well as other pharmacologic and biochemical studies.

Proposed Course:

(1) Biochemistry

(a) Nuclear extract.

We hope to be able to purify the protein(s) which specifically binds to and breaks DNA in the presence of intercalators. We are presently assessing our

degree of purification using SDS-polyacrylamide gel electrophoresis. We hope to characterize this activity (K_m, V_{max}, etc.) as well as identify requisite substrates and cofactors. Further purification may require hydroxylapatite chromatography and/or HPLC techniques.

(b) SV-40, in vitro.

A completely reconstituted system with pure protein plus defined substrate should allow us to characterize the site of DNA binding and breaking. It is then hoped that purification of these defined DNA's following reaction with m-AMSA and the nuclear extract protein could generate DNA which could be examined by restriction enzyme analysis and possibly DNA sequencing gels.

(c) SV-40 in vivo.

We will also use the techniques listed above in an attempt to characterize the DNA break sites produced within SV-40 DNA in vivo. Additionally, a quantitative relationship could be sought between the m-AMSA effects on the DNA of the SV-40 minichromosome and effects on the DNA of uninfected CV-1 cells. A marked difference between the frequency of m-AMSA-induced scission in the 2 DNA targets might suggest some intrinsic differences between them, (e.g.-chromosomal organization) either structural or biochemical. Alternatively, an equal break frequency in the 2 targets would suggest the processes breaking each DNA target are identical and probably independent of chromatin structure which is likely to be of different complexities in the 2 targets.

(d) Various sources of the m-AMSA-induced DNA bound protein have been sought. Perhaps our most promising is the nuclear scaffold prep of Laemmli. Further efforts in this vein are possible in an attempt to identify the form or, at least, molecular weight of the m-AMSA-induced DNA-bound protein and to compare it with that of the nuclear extract. We might anticipate only a portion (?subunit) of the protein within the extract will actually be covalently DNA-bound.

(e) Finally, an effort has begun to cleave DNA bound to polyvinyl chloride filters by m-AMSA-induced proteins so as to isolate relatively small segments of DNA to which the protein is bound for hybridization with specific ^{32}P-labeled probes such as those for specific gene sequences. This work has also just begun. It is anticipated that if successful, this technique will be used to identify whether intercalator-induced, DNA-protein crosslinks are selectively localized to particular DNA sites such as actively-transcribing gene sequences. (Also see under Cellular Biology).

(2) Cellular Biology

(a) The Ara-C and HU systems will be among the first in which our biochemical techniques will be applied. Are the drugs altering chromatin sites for m-AMSA, or altering enzyme binding sites or perhaps increasing the amount of enzyme? Further, how will these results aid us in more precisely characterizing the cell cycle biochemically?

(b) The results with 5-azacytidine suggest that alteration in DNA methylation may produce a DNA target with an altered susceptibility to intercalator-induced, protein-associated DNA scission. This altered susceptibility could result from the ability of DNA's possessing different degrees of cytosine methylation to have various degrees of the Z-rather than B-conformation. The abilities of drugs to intercalate and/or the putative DNA breaking protein to act may be different within DNA's of different conformation. The ability to obtain DNA plasmids of defined sequence and structure (Nordheim and Rich, PNAS 80: 1821, 1983) plus our ability to reconstitute our sysytem in vitro may allow us to explore directly the relationship between the intercalator-induced DNA scission and the 3-dimensional structure of the target DNA.

(c) Our work with the human breast cancer cells will focus on determining whether E_2-effects on cell growth rate or distribution within the cell cycle can be eliminated as explanations for our results. Techniques have been worked out to isolate each effect from the others and these experiments have already begun. The effects of E_2 on intercalator-induced cytotoxicity will also be performed in the MCF-7 line to see if enhanced intercalator-induced DNA scission affects cell survival following intercalator treatments. Additionally, we are examining the DNA of hormonally-responsive rat pituitary cells (GH_3) for its susceptibility to intercalator-induced scission following treatment of the cells with E_2 or dexamethasone. This may be a crucial study. GH_3 cells will transcribe the prolactin gene in response to E_2 and the growth hormone gene in response to dexamethasone. cDNA's for each gene are available. Most interestingly, the DNA's of these genes when actively transcribing possess different structures as analyzed by nuclease sensitivity (Levy-Wilson, Nucl. Acid Res., 11: 823, 1983). This cell line may allow us to test whether our findings with E_2 in MCF-7 were unique. If not, it may suggest whether the enhanced nucleuse sensitivity of actively-transcribing chromatin is identical with enhanced susceptibility to intercalator-induced scission.

If this is true, we may be able to test whether the additional DNA bound to filters following hormone plus intercalator treatment in hormonally-responsive cells is enriched for specific gene sequences. This study, or similar ones, may allow us to connect alterations in intercalator-induced DNA binding at specific DNA sites with alterations in intercalator effects on specific cell functions, e.g. gene expression.

(d) A similar approach may be taken in HL-60 cells as in GH3 cells. HL-60 are human leukemia cells capable of differentiating to granulocytoid cells following exposure to DMSO or to monocytoid cells following exposure to phorbol esters. During differentiation, the c-onc gene, c-myc, decreases its expression. We may explore whether intercalator-induced scission and crosslinking is altered during the course of HL-60 differentiation. If so, we could use the v-myc-^{32}P probe to see if the filter-bound DNA displays an altered proportion of this gene within the total DNA. The recent finding that the phorbol ester receptor and protein kinase C copurify (Niedel et al., PNAS 80: 36, 1983) and that among the phosphorylated substrates are

histones suggests a manner in which differentiating agents could produce alterations in cellular chromatin structure, through modification of chromosomal proteins.

(e) The relationship between intercalator-induced DNA scission and mutagenicity and sister-chromatid exchange production is also being sought in conjunction with Dr. Matthews O. Bradley of the Merck Institute for Therapeutic Research. Again, this may allow us to quantitatively relate intercalator effects on the DNA level with drug effects upon whole cell populations.

(f) In summary, our proposed course has three major goals: (1) to isolate the intercalator-related DNA breaking and binding protein using conventional biochemical techniques and a completely in vitro reconstituted system to quantify enzyme activity, (2) to employ DNA's of defined sequence within the reconstitution system to identify specific DNA scission sites and characterize the DNA substrate biochemically as well as to compare these results with those obtained on a defined DNA exposed to drug in vivo, and (3) to use this information plus the tools of modern molecular biology to identify specific sites of intercalator effects within cells and relate these effects to various aspects of cell physiology such as differentiation, gene transcription, cell cycle kinetics, mutagenicity and clastogenicity.

Publications:

1. Zwelling, L.A., and Mattern, M.R.: DNA repair deficiencies do not affect intercalator-induced cytotoxicity or DNA scission in human cells. Mutat. Res. Letts. 104: 295-304, 1982.

2. Zwelling, L.A., Kerrigan, D., and Michaels, S.: Cytotoxicity and DNA strand breaks by 5-iminodaunorubicin in mouse leukemia L1210 cells and a comparison with adriamycin and m-AMSA. Cancer Res. 42: 2687-2961, 1982.

3. Zwelling, L.A., Kerrigan, D., Pommier, Y., Michaels, S., Steren, A., and Kohn, K.W.: Formation and resealing of intercalator-induced DNA strand breaks in permeabilized L1210 cells without the stimulated synthesis of poly-(ADP-Ribose). J. Biol. Chem. 257: 8957-8963, 1982.

4. Zwelling, L.A., Kerrigan, D., Michaels, S., and Kohn, K.W.: Cooperative sequestration of m-AMSA in L1210 cells. Biochem. Pharmacol. 31: 3269-3277, 1982.

5. Zwelling, L.A., Michaels, S., Kerrigan, D., Pommier, Y., and Kohn, K.W.: Protein-concealed DNA strand breaks produced in mouse leukemia L1210 cells by ellipticine and 2-methyl-9-hydroxyellipticinium. Biochem. Pharm. 31: 3261-3267, 1982.

6. Kohn, K.W., and Zwelling, L.A.: Consequences of DNA intercalation: protein-associated DNA strand breaks. In Muggia, F.M., Young, C.W., and Carter, S.K. (Eds.): International Symposium on Anthracycline Antibiotics in Cancer Therapy. New York, Martinus, Nishoff, 1982, pp. 86-89.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06157-01 LMPH

PERIOD COVERED
October 1, 1983 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Use of an ELISA Assay for Detecting Pt-DNA Adducts

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Leonard A. Zwelling Sr. Investigator LMPH NCI

COOPERATING UNITS *(if any)*
LCCTP, DCBD
Medicine Branch, COP, DCT, NCI

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have recently shown that Pt-DNA adducts can be detected in murine malignant cells treated in vitro or in vivo with cisplatin using an ELISA assay. We are now seeking to quantify such adducts in the tissues of human subjects receiving cisplatin as anticancer therapy. The results of these assays will be compared with the antineoplastic and toxic effects of cisplatin within these patients.

PHS 6040 (Rev. 1/83) GPO 895-100

Project Description:

(1) The DNA of peripheral blood white cells from patients receiving cisplatin will be extracted and the frequency of Pt-DNA adducts will be determined by the ELISA assay developed by the combined efforts of Drs. Poirier, Yuspa and Zwelling along with Dr. Stephen Lippard of MIT.

(2) The results of these assays will be compared with the antineoplastic and toxic effects of cisplatin to determine if either of these effects relate to the level of DNA adducts in an accessible human tissue.

Methods Employed:

(1) ELISA assay of Poirier et al. (PNAS 79: 6443, 1982).

Major Findings:

(1) In the white cells of four normal volunteers and four patients receiving chemotherapy for the treatment of malignant lymphoma containing no cisplatin, no DNA-Pt adducts were detected. This was also true in five patients about to receive cisplatin.

(2) In 28 determinations of Pt-DNA adducts following drug administration, 8 were positive for the presence of Pt-DNA adducts. Thus, Pt-DNA adducts are detectable in human white blood cell DNA in vivo.

Proposed Course:

(1) Samples will continue to be collected (MB 179) from patients receiving single agent cisplatin (MB 176).

(2) Additionally, MB 165 (CHIPS) is a protocol for the therapy of advanced ovarian cancer in which cisplatin will be administered. We are planning to be included in the latest revision of this protocol so as to increase our experience with this assay as well as possibly obtain samples of ascitic and pleural fluid from these patients following cisplatin administration.

(3) Toxicity and efficacy comparisons will be made as further samples are obtained.

Publications:

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Symposium Series, 1983, pp. 27-49.

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ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1982 to September 30, 1983

The objectives of the Laboratory of Tumor Cell Biology are to develop, implement, and analyze data obtained from studies of cellular proliferation, cell differentiation, and biochemical growth characteristics of normal and malignant mammalian cells both in vivo and in vitro. Particular attention is given to hematopoietic cells, their normal behavior and especially changes seen during leukemogenesis. Because of unusual access to human blood cells and because of the interest of this group, there is special focus on human leukemias and lymphomas, and acquired immune deficiency syndrome. It is anticipated that an enhanced understanding of cell regulatory mechanisms will permit the optimal use of anti-tumor agents in the therapy of cancer and the development of new approaches.

The Laboratory of Tumor Cell Biology is concerned with several biological and biochemical problems: (1) Studies on the cellular and molecular origin and pathogenesis of human leukemia. Biochemical control mechanisms involved in cell differentiation and neoplastic transformation are examined. Tumor viruses of animals are used both as tools (to define and isolate genes and gene products important for growth in man) as well as for help in understanding mechanisms of naturally occurring animal leukemias. Also, studies designed to determine the distribution of human T cell leukemia virus (HTLV) in T cell leukemia patients, patients with acquired immune deficiency syndrome (AIDS), and normals in different parts of the world are being intensively studied. (2) Studies on the biochemical events preceding mitosis. An understanding of these events appears essential to the control of proliferation, information derived from such studies may lead to more effective inhibitors or neoplastic cell growth. Events leading to mitosis are also of interest since many of the effective antitumor agents are useful only when cells are in DNA replication or in mitosis. Phytohemagglutinin stimulated human lymphocytes and tissue culture cells are the principal tools in these studies. (3) Attempts to develop new approaches to cancer chemotherapy using information gained from basic cellular studies. (4) Studies on the development of biochemical nd immunological markers for malignant cells are carried out. Biochemical and immunological studies are also conducted in individuals with disorders associated with an increased incidence of neoplasia. (5) Controls regulating cellular growth and differentiation, and the process of malignant transformation in hematopoietic cells. (6) Growth factors (and their receptors) that control the growth and differentiation of blood cells have been isolated and are under intensive study, e.g., T-cell growth factor (TCGF), CSF, and related hematopoietic growth effecting molecules.

During the past year a number of findings were reported by investigators from the Laboratory.

Major Findings:

1. Partial amino acid sequence of purified human T cell growth factor (TCGF) has been determined.

2. Twenty new neoplastic human T-cell lines have been developed from patients with T cell malignancies and acquired immune deficiency syndrome (AIDS).

3. HTLV has been transmitted into cord blood lymphocytes, bone marrow and adult peripheral blood T cells.

4. Seroepidemiology studies show that HTLV is widespread in certain areas of Japan, West Indies, South East U.S.A., China, Russia, Africa, Malaysia, Central and South America.

5. Seroepidemiological studies show that HTLV antibodies are present in some patients with acquired immune deficiency syndrome (AIDS).

6. Amino acid sequence analysis of p24 and p15 has been completed. There are some similarities in the amino acid sequence of HTLV p24 and BLV p24 and HTLV p15 and BLV p12.

7. Cloning and chromosomal mapping of four human cellular onc genes has been completed.

8. Cloning of HTLV-I and HTLV-II genomes and flanking cellular sequences from cells of several T cell leukemia patients has been accomplished.

9. Molecular epidemiology survey for HTLV in human tumors has been carried out and shows the presence of HTLV provirus in a number of tumor tissues.

10. Nucleotide sequencing of HTLV-I LTR has been completed.

11. Cloning of a gene, HT-3, that is specifically transcribed in HTLV infected T cells has been accomplished.

12. Cloning of the gene for TCGF has been accomplished.

13. Cloning of genes from ALL and AML cells that transform mouse fibroblast cells in vitro has been carried out.

14. A differentiation inducing activity (DIA) has been shown to act synergistically with retinoic acid in inducing differentiation of fresh cells from patients with acute promyelocytic leukemia.

15. Retinoic acid induces the synthesis of NAD^+-glycohydrolase (NADase) in HL-60 cells. Other inducers of differentiation such as dimethylsulfoxide, hypoxanthine, 1, 25-dihydroxyvitamin D_3, and butyric acid do not induce this enzyme.

16. The induction of NADase has been adapted as a very sensitive and rapid assay for retinoids that induce differentiation of HL-60.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06117-11-LTCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert C. Gallo, Chief, Laboratory of Tumor Cell Biology, NCI

COOPERATING UNITS *(if any)*

Stu Aaronson, Viral Carcinogenesis Branch, National Cancer Institute; Rolf Neth, University of Hamburg; Robin Weiss, Imperial Cancer Research Fund, London, England; Dani Bolognesi and Bart Haynes, Duke University; Ken McCredie, M. D.

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION Sections on Hematopoietic Cellular Control Mechanisms, Hematopoietic Cell Biochemistry and Immunology, and Molecular Genetics of Hematopoietic Cells.

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
52	31	21

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells, designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for and cloning of viral genomes and genome products in human tumor tissues; (2) the identification, isolation and demonstration of biological activity of viral information in human leukemic cells and cells from patients with acquired immune deficiency syndrome (AIDS); (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation in vitro. (This relates to a major interest of the Laboratory: Does the phenotypic abnormality of leukemia in man result from a block in leukocyte maturation?) (5) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Prem S. Sarin	Chemist	LTCB NCI
Theodore Breitman	Chemist	LTCB NCI
Carl Saxinger	Microbiologist	LTCB NCI
Flossie Wong-Staal	Microbiologist	LTCB NCI
Michael Clarke	Clinical Associate	LTCB NCI
Edward Gelmann	Clinical Associate	LTCB NCI
John Horneff	Clinical Associate	LTCB NCI
Martha Michalski	Clinical Associate	LTCB NCI
Leonard Seigel	Clinical Associate	LTCB NCI
Suresh Arya	Cancer Expert	LTCB NCI
Joseph Gootenberg	Cancer Expert	LTCB NCI
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Francis Ruscetti	Cancer Expert	LTCB NCI
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Mikulas Popovic	Visiting Associate	LTCB NCI
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Masue Imaizumi	Visiting Fellow	LTCB NCI
Jorg Jendis	Visiting Fellow	LTCB NCI
Jorg Schupbach	Visiting Fellow	LTCB NCI
Anna Aldovini	Guest Worker	LTCB NCI
Carla Grandori	Guest Worker	LTCB NCI
Beatrice Hahn	Guest Worker	LTCB NCI
Stephan Lindner	Guest Worker	LTCB NCI
Beatrice Macchi	Guest Worker	LTCB NCI
Ishamel Reed	IPA	LTCB NCI

COOPERATING UNITS

Anderson Hospital and Tumor Institute; Bill Hardy, Sloan Kettering, New York; George Vande Woude, National Cancer Institute; Gianmarco Corneo, University of Milan; Yohei Ito, University of Kyoto; Umberto Torelli, University of Modena; Max Essex, Harvard University; Bill Haseltine, Harvard University; Jack Strominger, Harvard University; Henry Kaplan, Stanford University; Luc Montagnier, Pasteur Institute, Paris; Roger Monier, Cancer Institute, Villejuif; Volker Erfle, Munich; Armand Tavitian, Hospital St. Louis, Paris; Kendall Smith, Dartmouth Medical School; Ron Herberman, National Cancer Institute; Fernando deNoronha, Cornell University; Ivor Royston, University of California at San Diego; Bill Blattner, Epidemiology Branch, National Cancer Institute; Mark Smulson, Georgetown University; Isaac Witz, Tel Aviv University.

Projection Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia. Special focus is on the leukmias and lymphomas.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

   a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained, i.e., is their structure specific enough that we can use this information to find viral RNA in cells?

   c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

2. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization, restriction enzyme analysis and gene cloning.

3. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells were recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to

understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

5. Markers: (a) Immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells are being developed. (b) Techniques for detecting viral specific nucleic acids in intact cells are also being developed.

6. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type-C RNA tumor virus related markers and other biological markers.

7. Techniques are being developed to use monoclonal antibodies, prepared against cell surface antigens for subtyping and separation of peripheral blood and bone marrow cells with the help of a fluorescence activated cell sorter.

8. Antibodies associated with the membranes of human leukemic cells have been isolated. They are under study as to which protein antigens they interact with i.e., are they leukemia cell specific, e.g., viral, etc.

9. Recombinant DNA technique is being utilized to obtain molecular DNA clones of defective and non-defective primate and human viruses. DNA from these clones will be utilized to carry out transfection experiments and for generation of subgenomic fragments for probes and functional analysis.

10. Human T cell growth factor (TCGF) has been purified to homogeneity for further characterization. Studies are in progress to determine receptors on activated T cells for TCGF.

11. The distribution of T cell leukemia virus (HTLV) in human T cell leukemia patients and patients with acquired immune deficiency syndrome (AIDS) from various parts of the world is being actively pursued.

Major Findings

1. Partial amino acid sequence of purified human T cell growth factor (TCGF) has been determined.

2. Twenty new neoplastic human T-cell lines have been developed from patients with T cell malignancies and acquired immune deficiency syndrome (AIDS).

3. HTLV has been transmitted into cord blood lymphocytes, bone marrow and adult peripheral blood T cells.

4. Seroepidemiology studies show that HTLV is widespread in certain areas of Japan, West Indies, South East U.S.A., China, Russia, Africa, Malaysia, Central and South America.

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16. The induction of NADase has been adapted as a very sensitive and rapid assay for retinoids that induce differentiation of HL-60.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanism and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effect on Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72; Proc. Nat. Acad. Sci. 69: 2879-2884, 1972; Proc. Nat. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972).

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973; Biochim. Biophys. Acta 454: 212-221, 1976, 479: 198-206, 1977, 564: 235-245, 1979; Virology 112: 355-360, 1981).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique developed by Paran and Sachs. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units, focus formation and plaque assays for virus are carried out by conventional techniques.

7. Molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter techique with RNA covalently attached (Proc. Nat. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (e) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (XC test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using ficoll-hypaque gradients, sucrose density gradients, free flow electrophoresis and centrifugal elutriation. (Lancet 1: 508-509, 1976).

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SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1982 - September 30, 1983

I. General Organization

The Cancer Therapy Evaluation Program (CTEP) is responsible for the administration and coordination of the majority of the extramural clinical trials supported by DCT. These programs include the activities of the Clinical Cooperative Groups, the Phase I and Phase II new drug development contractors, and the holders of investigator-initiated grants (R01 and P01) relating to cancer treatment. Certain programs in developmental radiotherapy, such as high LET radiation, are administered in the Radiation Research Program. The Phase I development of biologic response modifiers is handled by the Biological Response Modifiers Program. During most of FY '83, CTEP was composed of two branches.

The Investigational Drug Branch (IDB) is responsible for sponsoring trials of new investigational drugs and of evaluating them for efficacy and toxicity. It does this by: 1) Obtaining Investigational New Drug exemption (IND) authorization from the Food and Drug Administration (FDA); 2) Coordinating and monitoring the Phase I trials of new agents developed by the DCT; 3) Planning with members of the Clinical Investigations Branch (see below) overall strategies for activity (Phase II) studies in specific tumor types; 4) Monitoring the results of the clinical trials; 5) Ensuring that clinical investigators using investigational new drugs are in compliance with federal regulations regarding the use of such agents; 6) Regulating the distribution of investigational new drugs for which NCI is the sponsor; 7) Maintaining close contact and ongoing dialogue with the pharmaceutical industry in an attempt to ensure that new drug development proceeds in a coordinated way.

The Clinical Investigations Branch (CIB) is responsible for clinical studies conducted under the Cooperative Agreement mechanism and those Phase II/III trials done under contract. It manages the clinical oncology and nutrition portfolios of R01 and P01 grants.

The Office of the Associate Director (OAD) integrates the efforts of the Branches. This goal is accomplished by weekly staff meetings, in which issues of concern to the program are discussed with the full staff, and by weekly branch chief meetings, where issues are further defined and decisions are made. The process of protocol review is administered within the OAD, by a central Protocol Office. The Protocol Office is also the receipt point at NCI for all protocols entered into the PDQ system. The OAD is responsible for overall program supervision and budgetary allocation.

II. Organizational and Personnel Changes

During the past year, Dr. Robert E. Wittes joined the staff of DCT as Associate Director, CTEP. Dr. William DeWys, who left CIB to become the Associate Director, Prevention Program, DRCCA, was replaced as Branch Chief by Dr. Michael Friedman. During much of 1983, Dr. Edwin M. Jacobs served as Acting Branch Chief, CIB. Dr. Silvia Marsoni was named Acting Deputy Chief, IDB. Dr. Brian Leyland-Jones was recruited to become Acting Chief, Drug Evaluation and Reporting Section, IDB. Dr. Luz Hammershaimb was named Acting Chief, Quality Assurance and Compliance Section, IDB. Dr. Wallace Williams and Ms. Joan Mauer were also added to the staff of that section.

Toward the end of the past year, the Biometric Research Branch was transferred from the Clinical Oncology Program to CTEP. The addition of Dr. Richard Simon and Dr. Susan Ellenberg to CTEP staff serves to increase the full-time presence of statistical expertise within the program.

III. Highlights in Program Development

A) Immunodeficiency Syndrome (AIDS)

During the past year, CTEP staff has been heavily involved in the development of an RFA for support of AIDS research, the expediting of the initial review process, formulation of funding recommendations, and the coordination of an Extramural Working Group. Thus far, a total of twelve institutions have been funded from this RFA (four from NIAID funds, eight from NCI funds) at an estimated annual total cost of over $3 million per year. Investigators supported by this mechanism are concentrating on a host of issues likely to be important in the elucidation of the pathogenesis of the AIDS epidemic; these include epidemiological approaches, both in the U.S. and in the Caribbean, further dissection of the immunologic lesion, a search for viral particles in material from AIDS patients, further characterization of the opportunistic infections which occur in this setting, the development of potentially useful animal models, and approaches to therapy.

B) Drug Development

During the past year, five investigational drugs sponsored by DCT were introduced into Phase I clinical trials; these include: 2-fluoro-ARA-AMP, tiazofurin, spiromustine, acodazole, and SR-2508. Four other drugs (caracemide, menogarol, taxol, and rapamycin) are expected to enter Phase I trials before the end of the year. Several agents or combinations of drugs introduced last year are continuing Phase I evaluation; these include CBDCA, dihydro-5-azacytidine, homoharringtonine, N-methylformamide, tricyclic nucleoside, echinomycin, cyclophosphamide + misonidazole, ARA-A + DCF, and bisantrene by continuous infusion.

NCI-supported Phase II trials have confirmed activity for several new agents. Mitoxantrone and bisantrene appear to have substantial activity in breast cancer. Mitoxantrone is also active in leukemias, lymphomas, and hepatomas. AZQ has produced objective responses in primary and secondary brain tumors as well as in lymphomas. Since AZQ has thus far exhibited only myelosuppression as a dose-limiting side effect, several investigators are interested in pursuing trials

with this agent at high doses together with autologous bone marrow protection. Spirogermanium has shown encouraging results in the lymphomas. 2'Deoxycoformycin, a drug whose initial evaluation was beset by problems with toxicity, has shown interesting levels of activity in T-cell lymphomas and leukemias, as well as chronic lymphocytic leukemia, in doses which appear to be quite tolerable. Dichloromethotrexate has substantial activity in carcinomas of bladder, cervix, and head and neck origin; current efforts with this agent involve its intra-arterial administration in selected cases, as well as combination treatment with cisplatin.

As in the past, CTEP staff has continued to work closely with representatives of the pharmaceutical industry to ensure that drug development within the clinical trials network supported by NCI proceeds in an orderly and systematic manner. Joint efforts with Lederle Laboratories resulted in the design and implementation of an important trial in patients with advanced breast cancer; the Southwest Oncology Group is studying the relative efficacy of bisantrene, mitoxantrone, and doxorubicin in this patient population. DCT staff, together with representatives of Bristol Laboratories, have devised a comprehensive strategy for the study of two new platinum analogs within the Cooperative Groups and Phase II/III contractors. Discussions with representatives of Farmitalia Carlo Erba are expected to result in similar strategies for the study of anthracycline derivatives during the next year.

During the past year, IDB staff began distribution of the CTEP Letter, an informational newsletter which is regularly mailed to the Cooperative Group Chairmen and Phase I and II contractors. The CTEP Letter contains information on the drugs entering Phase I and Phase II trials. In addition to pertinent preclincal data, the "Letter" discusses important adverse drug reactions. It also outlines the scope of ongoing trials and areas in which further study is needed. Thus far, sixteen drugs have appeared in the CTEP Letter. Response by extramural investigators has been overwhelmingly favorable.

C) Monitoring of Clinical Trials

During the past year, the site visit monitoring system has been expanded in all NCI-supported clinical activities. For Cooperative Group members, site visits will be made on the average of once every three years, though each member will be at risk for a visit at any time. Affiliate group members will also be site visited; initially all affiliates will be visited within the first 12-18 months of the affiliate monitoring program. Major attention during these monitoring site visits will be devoted to data accuracy, quantitative accountability for experimental drug use, and compliance with federal regulations regarding Institutional Review Board approval for experimental protocols and for informed consent. Also included in the overall program of site visit monitoring are the cancer centers, the CCOP's, and R01 and P01 supported trials.

During the past year, a formal system of review of informed consent documents was implemented as part of CTEP's protocol review. In parallel with scientific review of the protocol itself, the associated informed consent document is reviewed for inclusion of the essential elements of informed consent mandated by federal regulations. No protocol is approved unless the informed consent meets these criteria.

During the past year increasing attention was devoted to the analysis and process of adverse drug reactions involving experimental agents; approximately 182 such reactions were evaluated and reported to the Food and Drug Administration by CTEP. This represents an impressive increase over previous years and is almost certainly a result of the extensive interaction with the extramural community about the importance of reporting such reactions, even when their connection with the experimental drug is suspected but not certain. The extramural community has been repeatedly advised of the NCI policies regarding reporting of adverse drug reactions, most recently in January, 1983.

To ensure that experimental drugs are used for their intended purpose, a tighter system of drug accountability was implemented during the past year. In February, 1983, an instruction manual was sent to about 5,000 investigators detailing NCI policy on drug accountability and outlining the procedures for accounting for investigational drugs on a patient-by-patient basis. An audit of these drug logs at all participating institutions is an important part of the site visit monitoring system (see above).

D) Surgical Oncology

Recognizing the importance of developing a trained and committed cadre of investigators in surgical oncology for clinical cancer research, the DCT has proceeded along several lines. Funds will be committed to ensure that up to eight qualified surgical oncologists in training may be given Physician Investigator Development Awards (K08) for research training in basic or clinical sciences relating to surgical oncology. Surgical oncology has been added to the group of specialties to which the Professional Oncology Education Program (R-25) has been targeted; these are awards made to institutions to strengthen clinical teaching to medical and nursing students in designated areas. Supported in this way, institutions which are already strong in surgical oncology may be able to reach bright, young medical students during a formative period of their development and influence career choices. The program announcement in surgical oncology, issued in June 1981 has been revised to make its intent clearer; we expect that it will be issued four times during the next year. DCT also plans to reissue the RFA for P20 planning grants in surgical oncology, pending availability of funds for this purpose.

E) Group Studies

1) NCI-Pan American Health Organization (PAHO) Collaborative Cancer Treatment Research Program - We have recently conducted a reevaluation of the importance of this resource to the National Cancer Program. This has resulted in: a) the formation of in internal scientific advisory committee to coordinate scientific directions, review and provide advice for protocols, and to review and evaluate membership status, b) a decision to concentrate primarily on Phase II trials involving diseases prevalent in Latin America - These would include cancers of the penis, vulva, cervix, esophagus, head and neck, stomach, and lung, c) careful assessment of the feasibility and scientific merit of large-scale Phase III trials prior to activation; these trials will preferably be performed by more than one center to ensure adequate accrual. We feel that with proper attention

to such matters the clinical trials conducted under this mechanism can make important contributions to knowledge about cancer treatment. Because of the difference in availability of patients with particular kinds of malignancies, such trials are difficult or impossible to do in North America or Western Europe.

2) Intergroup Testicular Study - This collaboration between seven Cooperative Groups and four large institutions with particular interest in testicular cancer is a randomized, controlled study of adjuvant chemotherapy in Stage II resectable disease; patients with Stage I disease are monitored, but not actively treated. The adjuvant study tests the relative efficacy of immediate adjuvant treatment with chemotherapy versus a policy of treatment upon relapse. During the past year, 181 patients were entered onto the Stage II study and 193 onto the Stage I observational study. This Intergroup Testicular Study constitutes impressive documentation that the intergroup mechanism is a workable means of performing an adjuvant study in a rare adult tumor.

3) The Leukemia Intergroup Study has as its major goal the delineation of laboratory parameters which may be useful as prognostic factors in determining optimal therapy. The group has concentrated on clinical, cell kinetic, and pharmacologic parameters. Early results suggest that such laboratory parameters can be generated in a multi-institutional setting and provide a framework for further efforts by Cooperative Groups along these lines.

4) During the past year, the Head and Neck Contract Group terminated accrual on a multimodality adjuvant study designed to explore the efficacy of initial treatment with chemotherapy, with or without subsequent maintenance chemotherapy, in patients with resectable epidermoid head and neck cancer who also received standard surgery and radiation therapy. This trial established that a complex therapeutic effort involving three modalities could be performed in these diseases in a multicenter setting with good accrual and excellent quality control. Efforts to continue intergroup activities in head and neck cancer, with the previous contract group as a nucleus, are currently in progress.

5) Clinical trials in the National Bladder and National Prostatic Cancer Projects in the Organ System Program have recently been transferred to DCT. These groups are both surgically based with excellent accrual capability and have conducted a wide variety of therapeutic trials in the respective diseases over the past several years. The shift in administration to DCT should allow for easier access to experimental agents and increasing scientific interchange with NCI staff.

6) The Nutrition Oncology Research Cooperative Agreement (NORCA) has recently been formed from the conversion of three previous contracts to Cooperative Agreements. The purpose of NORCA is to explore the role of nutritional substances in the treatment and/or support of the cancer patient. Composed of Emory University, Memorial Sloan-Kettering Cancer Center (with Brookhaven National Laboratories), and Toronto General Hospital (with Princess Margaret Hospital), the group is currently evaluating approaches to the assessment of lean body mass in cancer patients and methods of preserving lean body mass in face of a tumor load.

IV. Publications

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5. Gad-el-Mawla, N., Abul-ela, M., Mansour, M.A., Macdonald, J.S.: Preoperative chemotherapy in relatively advanced head and neck cancer. Am. J. Clin. Oncol. (in press).

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11. Kisner, D.L. and Macdonald, J.S.: Mitomycin-C in the treatment of gastric and pancreatic carcinomas. In Rozencweig, M. (Ed.): Mitomycin-C. Amsterdam, Excerpta Medica, 1982, pp 64-75.

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CLINICAL INVESTIGATIONS BRANCH (CIB)
Table of Contents

The Clinical Investigations Branch (CIB) is responsible for the scientific administration of the national cooperative clinical trials groups (the Cooperative Group Program); for scientific monitoring of the Phase II/III contracts, the disease-oriented contracts, an individual investigator-initiated clinical oncology grant program, a nutrition grant program, the interagency agreements, the surgical oncology grant program, and the Intergroup Testicular study; and for scientific administration of the Program Projects grants in clinical cancer treatment.

During this fiscal year the GITSG, LCSG, and NSABP contracts were converted to cooperative agreements while the cooperative group grants were converted in the prior fiscal year.

1.0 Personnel

1. Edwin Jacobs, M.D. - Acting Chief, CIB; Acting Head, Nutrition Section
2. Edwin Jacobs, M.D. - Associate Chief, CIB
3. Richard Ungerleider, M.D. - Head, Pediatric Section
4. John Y. Killen, Jr., M.D. - Head, Medicine Section
5. Ernest deMoss, M.D., M.P.H. - Head, Surgery Section
6. Mario Eisenberger, M.D. - Senior Investigator, Medicine Section
7. Freddie Ann Hoffman, M.D. - Senior Investigator, Nutrition Section
8. Elizabeth I. Read, M.D. - Senior Investigator, Medicine Section
9. Elaine Lewis - Acting Secretary to Acting Chief and Associate Chief
10. Jeannie Williams - Secretary to Drs. deMoss and Hoffman
11. Sandra Chartier - Secretary to Drs. Killen, Read, and Eisenberger
12. Helen Goldberg - Secretary to Dr. Ungerleider, and Medicine Section
12. Helen Bradley - Stay-in-School, CIB
13. Charles Pruet, M.D. - Guest Worker, Surgical Oncology

Dr. Jacobs is responsible for the overall administration of the Branch and coordination of its activities with the Cancer Therapy Evaluation Program, the Grants Administration Branch, the Cancer Clinical Investigations Review Committee (CCIRC), the National Cancer Advisory Board, and the Cancer Regional Studies Review Committee (CRSRC). He also supervises the Project Officers on clinical contracts, and the Program Directors on grants and cooperative agreements. He is Acting Head of the Nutrition Section, CIB, and coordinator of an intergroup protocol for Stage I and II testicular cancer.

Dr. Jacobs serves as Associate Chief of the CIB and Program Director for the Clinical Cooperative Group Program (U10's). He coordinates the program review with the Executive Secretary of the CCIRC, and the Executive Secretary of the CRSRC for the cooperative agreements. He is administrator of the cooperative clinical group protocols, except for the regional group studies. He is also the Co-Project Officer for the MSKCC Phase II/III contract and serves on the Protocol Data Query Editorial Board.

Dr. Ungerleider is Head of the Pediatric Section, CIB, and assists the Program Director in the administration of the R01 grants for pediatric cancers. He is Program Coordinator for the Cooperative Groups which are conducting studies of pediatric cancers. He also serves on the Protocol Data Query Editorial Board.

Dr. Killen is Head of the Medicine Section, CIB. He is Program Director for the R01 Clinical Treatment Grants, and the Regional Group cooperative agreements. He is the Project Officer for the Emmes Corporation Statistical contract, and the Gastrointestinal Cancer Study Group contract. Also he serves as liaison officer to the EORTC Protocol Review Committees and he is the Chairman for the NCI/NIAIDS Extramural Working Group on Immunodeficiency Syndrome (AIDS).

Dr. deMoss is Head of the Surgery Section, CIB, the Project Officer for the Head and Neck Contract, the P20, and R01 Surgical Oncology grant programs. He also participates in the Surgery Clinic in the Clinical Center, N.I.H.

Dr. Eisenberger is the Program Director for the P01 Program Project grants, and coordinates review of these grants with the Clinical Cancer Program Project Review Committee. He is also liaison officer for the NPCP and the NBCP. He is Project Officer of the PAHO and the Phase II/III contracts.

Dr. Read is Program Director for the R01 grants. She also is Project Officer for the Milano Breast Cancer contract and the Bone Marrow Transplant Registry. She is the liaison officer with the Breast Cancer Task Force.

Dr. Hoffman is Program Director for the R01 Nutrition Program and the U10 Cooperative Agreements in Nutrition. She is also Program Director for Clinical Treatment R01 grants dealing with Supportive Care. She is currently Project Officer for Nutrition contracts and the Immunotherapy contracts.

Dr. Richard Simon and Dr. Susan Ellenberg divide their time between participation in protocol review and general consultation on CTEP and other DCT projects. They are principal reviewers on the Phase III and many of the Phase II protocols. They also serve as liaison between CTEP and the cooperative group statistical centers.

Ms. Elaine Lewis as secretary to Dr. Jacobs acts as administrative secretary for the Branch and assists Dr. Jacobs in the administration of U10 grants and coordinates the review of the cooperative group protocols.

Ms. Sandra Chartier is secretary to Dr. Killen, Dr. Read, and Dr. Eisenberger in the Medicine Section. Ms. Jeannie Williams serves as secretary to Dr. deMoss and Dr. Hoffman. Ms. Helen Goldberg is secretary to Dr. Ungerleider and to the Medicine Branch. Ms. Helen Bradley provides support to the CIB staff.

Dr. Pruet is stationed at the National Naval Medical Center, and provides expertise to the CIB in surgical oncology, particularly in head and neck surgery.

2.0 Grant Programs

2.1 Cooperative Clinical Trials Groups - Cooperative Agreements

The Cooperative Group Program was initiated by the Cancer Chemotherapy National Service Center to test the new agents from the NCI drug development program (1955-66). The program underwent several administrative changes and most recently has been in the Division of Cancer Treatment (1975 - present) where the major emphasis has been on combined modality approaches to cancer treatment. In March 1979, the DCT Board of Scientific Counselors conducted an in-depth review of the progress of clinical research supported through grants and contracts by the DCT. As a result of the analysis of this review, and the past history of DCT staff involvement and cooperation with the cooperative groups, the NCI decided to fund its research clinical trials program through cooperative agreements. Subsequently, the cooperative clinical trials groups were converted to this mechanism.

2.11 Listing of the Cooperative Clinicial Trials Groups

Multimodality Multidisease Groups
Cancer and Leukemia Group B (CALGB)
Eastern Cooperative Oncology Group (ECOG)
Mid-Atlantic Oncology Group (MAOP)
North Central Cancer Treatment Group (NCCTG)
Northern California Oncology Group (NCOG)
Piedmont Oncology Association (POA)
Southeastern Cancer Study Group (SEG)
Southwest Oncology Group (SWOG)

Multimodality Groups Devoted to a Major Oncologic Area
Childrens Cancer Study Group (CCSG)
Gynecologic Oncology Group (GOG)
Pediatric Oncology Group (POG)
Lung Cancer Study Group (LCSG)

Single Modality Group
Radiation Therapy Oncology Group (RTOG)

Single Disease Groups
Intergroup Ewing's Sarcoma Study (IESS)
Intergroup Rhabdomyosarcoma Study (IRS)
National Surgical Adjuvant Breast and Bowel Project (NASBP)
National Wilms' Tumor Study Group (NWTSG)
Polycythemia Vera Study Group (PVSG)
Radiotherapy Hodgkin's Disease Group (RHDG)
Determinants of Response in Acute Myelocytic Leukemia Group (AML)

Special Activities Groups
European Organization for Research on Treatment for Cancer (EORTC)
  Operations and Statistical Office
Lymphoma Patholgoy Reference Center (LPRC)
Radiologic Physics Center (RPC) (see Radiation Research Program)
Quality Assurance Review Center (QUARC)
Nutrition Oncology Research Cooperative Agreement (NORCA)

2.12 Description of the Clinical Cooperative Group Program

The Cancer and Leukemia Group B (CALGB), founded in 1955, studied primarily hematologic malignancies until the 1970's when it also developed multimodal studies in solid tumors. It has made major contributions in the chemotherapy of breast cancer as well as the leukemias and lymphomas. In 1982 the group elected a new chairman and narrowed their scientific scope to the treatment of leukemia, lymphoma, lung, and breast cancer. The statistical office and operations office was moved from New York to Boston.

The Childrens Cancer Study Group (CCSG) is a multimodality organization concerned exclusively with pediatric malignancies. They have initiated major Phase II and III studies in hematologic and solid tumors, and have begun to collect information about the long-term effects of cancer therapy. They recently decided to give more emphasis to innovative pilot studies (e.g., bone marrow transplantation) among selected institutions while maintaining their interest in group-wide studies.

The Eastern Cooperative Group (ECOG), founded in the 1950's, developed and remains committed to multimodal solid tumor studies, but has increased studies in the hematologic malignancies. They have a strong surgical committee. The group has made major contributions in breast and gastrointestinal malignancies, It was a pioneer group in the development of a quality control monitoring program.

The Gastrointestinal Tumor Study Group (GITSG) began activity in 1974-1975 as a disease-specific contract-supported research group. Its primary mission has been and continues to be the evaluation of new surgical adjuvant therapies for gastrointestinal malignancy. The group also evaluates new treatments for advanced disease with the objective of identifying therapy worthy of evaluation in the adjuvant setting. The group has recently been converted to the cooperative agreement mechanism.

The Gynecologic Oncology Group (GOG) has involved the specialties of gynecology, radiation, medical oncology, and pathology for research in gynecologic cancers. They have done a systematic analysis of Phase II drug activity in several gynecologic malignancies as well as Phase III studies in early ovarian and cervical cancers.

The Mid-Atlantic Oncology Program (MAOP) began activity in 1982 as one of two Regional Clinical Trials Groups funded in response to an NCI RFA. The objectives of the group are to extend the latest developments in cancer therapy into the community through joint participation of physicians from private practice and academic centers in cooperative clinical research.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) is a pioneer multimodality group. In the past, it focused exclusively on primary treatment of breast cancer, but now it is also involved in studies of primary colorectal cancer. Major contributions to our theory and practice of adjuvant chemotherapy have been accomplished by this group. A current major study compares segmental mastectomy with or without radiotherapy to total mastectomy. Recently they developed protocols to study adjuvant chemotherapy in Stage I breast cancer.

The National Wilms' Tumor Study Group (NWTSG) is an intergroup organization incorporating the pediatric cooperative clinical groups along with several independent investigators. Their third study (NWTSG-3) is primarily concerned with refinement of therapy. NWTSG-1 and NWTSG-2 conclusively demonstrated that most children with this tumor can now be expected to survive if they are managed by combined modality therapy from the outset, and that prognosis is closely related to histopathologic findings. They are closely monitoring the late effects of cancer therapy in young children.

The North Central Cancer Treatment Group (NCCTG), organized in 1980, consists of the Mayo Comprehensive Cancer Center and ten clinics in the North Central region. The objectives of this Regional Clinical Trials Group is to make the most promising cancer research accessible to patients in their region, and to conduct clinical research of high quality in a community setting.

The Northern California Oncology Group (NCOG), a regional group organized in 1976, has developed programs in brain tumors, high LET radiation and radiosensitizer studies. They have participated in the head and neck contract, and have expertise in biological response modifiers and hyperthermia. Recently they elected a new Chairman and are refocusing their scientific directions.

Pediatric Oncology Group (POG) is a multimodality organization formed in 1980 from pediatric members of SWOG and CALGB. They have initiated new Phase II and III studies with a major interest in the classification of childhood leukemias through the use of cell markers. They have initiated a randomized trial of adjuvant therapy in osteogenic sarcoma.

The Piedmont Oncology Association (POA) was the first of two new Regional Cooperative Groups to be funded in 1982 as a result of an RFA issued by the NCI in 1981. Its center of operation is located at the Bowman Gray School of Medicine and its membership is composed largely of trained oncologists in private practice in the Piedmont area. The group's objective is to facilitate the dissemination of new developments in cancer therapy through participation in cooperative group research.

The Polycythemia Vera Study Group (PVSG), founded in 1967 has protocols to determine the natural history, course, and optimum therapy of polycythemia vera. Currently it is funded for followup and final analyses of their primary protocol which has shown an increase in incidence of leukemia in patients treated with chlorambucil as compared to radioactive phosphorous or phlebotomy.

The Radiation Therapy Oncology Group was formed in 1971 following a multi-institutional methotrexate study in head and neck cancer. Their protocols explore the methodology and technique of radiation therapy as applied in various tumor types, and disease-oriented studies exploring more than one modality of treatment using radiation therapy as a primary focus for study. Studies include time-dose relationships, the use of radiosensitizers, high LET radiation, hyperthermia, and a study of the late effects of radiation therapy. The group elected a new Chairman in 1980. The statistical operation office was transferred from Boston to RTOG Headquarters in Philadelphia and a new group statistician was appointed.

The Southeastern Cancer Study Group (SEG), previously involved in hematology studies, was reorganized in 1975 and has developed multimodal studies in lung cancer, melanoma and genitourinary cancers. They have initiated a new information system which utilizes microcomputers in clinical protocols. They have recently elected a new Chairman and are reorganizing some of their scientific directions.

The Southwest Oncology Group (SWOG), a pioneer group in clinical trials, continues to expand its activities and interests. One of the largest groups, with an annual accrual of about 3700 patients, it has the resources to rapidly complete Phase II and III studies. They have made significant contributions in AML, myeloma, lymphoma, and breast cancer. In 1981 the group elected a new Chairman, and are currently evaluating their scientific trends.

The Intergroup Ewing's Sarcoma Study (IESS), organized in 1973, is conducted by CCSG and POG members. It has accumulated the largest group of patients with Ewing's sarcoma in any study in the world. IESS-1 demonstrated that addition of either adriamycin or bilateral pulmonary irradiation to chemotherapy with VAC improves response and survival of children with nonmetastatic disease. IESS-2 has as its objective improved survival and relapse-free survival rates with the fewest long-term complications. Because of lack of new directions the IESS is on phase-out funding while innovative studies will be pursued by POG and CCSG independently.

The Intergroup Rhabdomyosarcoma Study Group (IRS) is composed of members of POG and CCSG. Their first study developed a staging system, demonstrated varied prognoses depending on site, and evaluated the effect of multimodal therapy. Their current study has incorporated special treatment considerations relating to primary site of disease. A third study is currently being reviewed and will involve a trial of intensive multidrug chemotherapy verus total body irradiation with autologous bone marrow infusion.

The Radiotherapy Hodgkin's Disease Group (RHDG) has studied whether survival in localized Hodgkin's disease was different when patients received involved field of radiation, or extended fields. The trial is presently in followup.

The Lung Cancer Study Group (LCSG), converted to a cooperative agreement, is composed of six institutions supported by a central pathology and statistical center studying potentially resectable non-oat cell lung cancer. In plans for the future, one protocol is proposed as a replacement study while four new protocols are an extension of LCSG's previous work. One study combines therapies to examine surgical, chemotherapeutic and radiotherapeutic approaches. Another examines a different sequence of therapy, by studying adjuvant therapy used prior to surgery in an attempt to convert patients from non-resectable to resectable. An additional study will examine the role of surgery in SCLC. However, with the increased intensity of treatment, a significant quality of life issues have arisen and will be assessed in a study.

The Nutrition Oncology Research Cooperative Agreement (NORCA) has recently organized following conversion of three contracts to cooperative agreements, to explore the role of diet, nutrition and nutrients in the treatment and support of the cancer patient. This three-institution group, which includes Emory University, Memorial Sloan Kettering Cancer Center and its affiliate, Brookhaven Laboratories, and Toronto General Hospital and its affiliate, Princess Margaret Hospital in Toronto, is currently conducting a nutritional assessment and intervention protocol, evaluating newer approaches to the preservation and assessment of lean-body mass of cancer patients. This group is investigating many of the biochemical and metabolic alterations and nutritional complications resulting from malignancy and anti-neoplastic therapy.

The following special activities groups provide support services for groups:

The Operations and Statistical Office of the EORTC is funded by DCT. A representative of CTEP serves on its protocol review committee.

The Lymphoma Pathology Reference Center (LPRC) provides expert review of pathological material for the groups performing therapeutic research in malignant lymphoma.

The Quality Assurance Review Center (QUARC) provides radiation therapy quality control for three national cooperative groups (CCSG, CALGB, POG), and two pediatric intergroup studies (IRS, NWTSG).

2.13 Summary of Accomplishments

Phase I and broad Phase II trials comprised a substantial effort in the past, but now their emphasis has expanded to include Phase III and combined modality studies with curative intent (adjuvant studies).

Change in direction has been represented by group membership in specialties. There has been a steady increase in the groups' medical oncologists and pediatric oncologists over the years, and the large increase in numbers of pathologists, radiotherapists, surgeons, and other physicians in the past four years is impressive and is a direct reflection of the move toward the multidisciplinary clinical research of cancer. Innovative pilot Phase I and Phase II studies open to one or a few institutions continue to be a formal part of cooperative group programs.

Additionally, the cooperative groups serve as a research base for cancer control programs and to the new Community Clinical Oncology Program (CCOPS).

The scientific progress of the groups are reflected in publications of numerous papers and abstracts which are listed as part of the progress reports. Some specific areas where noteworthy contributions have been made include:

1) Improved statistical methods for conducting clinical trials.
2) Definition of prognostic factors in childhood leukemia.

3) Progressively improved therapy in leukemia with improved survival.
4) Intergroup trials in Wilms' tumor widely extending the benefit of combined modality therapy and reduced side effects with more conservative therapy.
5) Delineation of the natural history of polycythemia vera and documented leukemogenic effects of chlorambucil in PCV.
6) Large scale adjuvant trials in operable breast, colon, and rectal cancer which should define the role of long-term chemotherapy of micrometastases.
7) Large scale trials of combined modality therapy of small cell lung cancer and new studies of surgery as an adjuvant.
8) Conducted studies where exaggerated results of preliminary studies were refuted by carefully done randomized multi-institutional trials.
9) Initiation of national intergroup mesothelioma and sarcoma clinical trials of adjuvant adriamycin.
10) Initiation of intergroup (national) Stage I and II testicular cancer trial to study prognostic factors and adjuvant chemotherapy.
11) Developed pilot studies of in vitro techniques to assess activity of new agents in clinical cooperative groups.
12) Introduction of cell surface markers and other assay techniques in large clinical trials (eg. ER/PgR in breast; markers, monoclonal antibodies and cytogenetics in pediatric and adult ALL).
13) Initiation of protocols studying biological response modifiers.
14) Initiation of national intergroup surgical protocol in Stage I melanoma.
15) Developed pilot studies for use of new computer technology in clinical trials.
16) Establishing the role of tamoxifen in adjuvant therapy of breast cancer.
17) Establishing the optimum surgery in primary breast cancer and the role of radiotherapy.
18) Demonstrated the lack of usefulness of MeCCNU when added to 5-FU in colorectal cancer and established a definite incidence of leukemogenesis.
19) Prospective randomized Phase II studies of new agents in gynecologic malignancies.
20) Development of regional clinical trials groups.
21) Development of collaborative studies of high LET radiation, radiosensitizer and hyperfractionation.
22) Development of a study to establish the role of VP-16 in front-line combination chemotherapy in testicular cancer.
23) Integration of regional and community hospitals in cooperative group clinical trials.
24) Study of cardiotoxicity of adriamycin by endocardial biopsy in weekly adriamycin as compared to every three weeks.
25) Initiation of pathology review in group clinical trials in gynecologic cancers, lung cancers, sarcomas, melanoma and testicular cancer.
26) Demonstrated that children with non-orbital, non-parameningeal head and neck sarcomas, if treated in accordance with the IRS protocol, have an excellent rate of local control and survival.
27) Prolonged the second marrow remission in acute lymphoblastic leukemia to a median of 12 months in pediatrics.
28) Confirmed in a randomized prospective controlled pediatric clinical trial that cranial radiotherapy is not necessary to prevent CNS leukemia in good-prognosis newly diagnosed patients.

29) In a study with NCI demonstrated that very high dose intravenous MTX can replace both cranial irradiation and intrathecal MTX for prevention of CNS relapse in both immediate and poor prognosis acute lymphoblastic leukemia pediatric patients.
30) Proved that in general children with acute lymphoblastic leukemia who do not relapse within three years of diagnosis do not benefit in the long-term from two more years of therapy.
31) Implementation of an internal site visit audits to assess performance of member institutions. Improvement of quality in pathology, surgery and radiotherapy by establishing quality control committees.

2.2 Program Project Grants (PO1)

At the present time there are 34 active clinical program project grants. Program projects grants provide research support for broadly based programs that blend pre-clinical and clinical activities.

Each grant involves a number of investigators each of whom conducts a research project designed to elucidate one or more aspects of a common goal. These efforts are conducted in an organized fashion in order to facilitate the interactions of these participating investigators. This approach is designed to acquire knowledge more effectively than would a simple aggregate of research projects operating without organization and thematic integration.

Historically, the program has supported highly successful research projects that have made significant contributions. By bringing together basic and clinical investigators, the program has been able to provide excellent patient care and also explore basic elements in tumor biology.

Although clinical research is the main thrust of all programs, substantial efforts in more basic elements are present. These activities include drug development and pharmacology, cell kinetics, immunobiology, marrow transplantation, histopathology and hematology.

The development of potentially curative strategies based on investigation of the kinetic basis for drug responsiveness of common tumor types has been a high priority. A variety of new methods have been developed and some older techniques have been used in new or different ways to predict kinetic patterns.

The aggressive use of bone marrow transplantation as an adjunct to other forms of treatment continues. Monoclonal antibodies directed against human hemopoietic and immunologic precursors and leukemic cell antigens are being used as research tools. Progress has been made in efforts to define the cellular and clinical characteristics of malignant lymphomas and related leukemias in terms of T and B lymphocyte systems. These efforts have lead to new studies on control mechanisms in lymphoma induction and progression, membrane and cytoplasmic markers, and cell surface receptors and antigens.

In vitro assays using explanted tumor tissue in culture to measure the effectiveness of drugs and other types of treatment methods have been correlated with in vivo activity. Among the important program project grants are:

1) Rosenberg, et al have a multidisciplinary program project in Hodgkin's and non-Hodgkin's lymphomas ranging from clinical to preclinical areas. In the clinical section important trials are being conducted with the intent to improve or maintain the chances of curing these diseases at the cost of lesser toxicity. In the preclinical area are development of monoclonal antibodies, important biological aspects of the disease and basic immunological studies.

2) Santos, et al are involved in bone marrrow transplantation for acute leukemia at a large center. A conditioning program without radiotherapy has been uniquely successful with a theoretical advantage of lessening pulmonary toxicity attributable to total body irradiation used routinely by other centers.

3) O'Reilly et al has demonstrated the use of soy-bean lectin-based fractionation procedure for T cell depletion in bone marrow transplantation for severe combined immuno-deficiency disease.

4) Herbst et al in developmental studies in hormone receptors in gynecological malignancies have contributed significantly to the better understanding of the biology of these tumors and may provide important data to serve as background for treatment.

5) Morton, et al are involved in new surgical concepts featuring advanced pathological staging of primary malignancies and early identification of metastatic disease by immunological techniques involving measurement of serum and urinary tumor associated antigens, tumor specific serum antibodies and circulating immune complexes.

6) Frei, et al have continued to characterize subsets of lymphoid cells, both functionally and through the development of cell surface markers capable of defining functionally unique populations of T, B, and null cells. These markers have proven to be extraordinarily useful in the characterization of the immunologic function of populations of cells in man and provide the basis for the understanding of specific defects in cellular immune responses in patients with cancer, autoimmune, and immunodeficiency diseases.

A large variety of tumors and areas are integrated in all the currently active grants which are likely to provide important leads in the biology and treatment of cancer.

2.3 R01 Grant Programs

2.31 Clinical Oncology

Description

The purpose of this program is to support research involving treatment of cancer patients. The range of the projects supported includes basic and clinical studies which are directly or ultimately aimed at improving methods of cancer therapy, including chemotherapy, radiation therapy, immunotherapy, surgery and supportive care. While many of these studies concern basic mechanisms of anti-neoplastic agents in cells, animals, and humans, there is an emphasis on clinical studies which relate directly to human cancer treatment. Improved methods of experimental design and statistical methods for clinical cancer studies are an integral part of the research fostered by this program. The program currently includes 82 grants.

Accomplishments: Many preclinical and clinical studies are completed or ongoing. Several examples are:

1. Friedlander, et al, have established banks of cortical bone allografts at four major institutions, as well as a system for data collection on bone donors and recipients, as prerequisites for a large clinical study of transplantation of cortical bone. These transplants are being done as part of reconstructive orthopedic surgery in an attempt to spare limbs in patients who require resection of large bone tumors.

2. Honn has demonstrated that several compounds which inhibit thromboxane in platelets also inhibit the platelet aggregation response to several types of cancer cells. These studies are aimed to study the mechanisms by which various agents decrease the ability of tumors metastases to become established.

3. Joshi has found that progestins regulate the serum and endometrial PEP level in normal postmenopausal women, and that it is possible to monitor effects of exogenous progestins on human endometrium by serum or endometrium PEP analysis. These studies are being expanded to study the effects of progestin treatment on patients with endometrial cancer.

4. Rowley, et al, have demonstrated that chromosome abnormalities in bone marrow cells from patients with secondary acute leukemia occur in non-random patterns. Many of these leukemias have been associated with previous chemotherapy or radiation therapy for other cancers. Further studies of the mechanisms of chromosomal damage and their relationship to clinical characteristics of the leukemia are underway.

5. Von Hoff, et al, have further refined techniques for culturing human tumor stem cells and performing drug sensitivity testing on tumor cells from patients. The human tumor stem cells assay has become a major focus of basic research activity in clinical oncology, with great potential for both delineating certain aspects of tumor cell biology and for direct study of treatment effects on tumor cells from patients undergoing clinical therapy.

6. Kufe has demonstrated that 5-Fluorouracil cytotoxicity of human tumor cells is related to incorporation of this drug into RNA and DNA in human cancer cells. While attempts to modulate the activity of this antineoplastic agent in tumor cells has not yet been successful in clinical trials, further studies are expected to delineate more precisely the drug's mechanisms of action.

2.32 Cancer and Nutrition Program

Description

A separate grant program was created and an RFA released in September 1979. Grants supported by this program include both preclinical and clinical studies encompassing areas such as pathophysiology of cancer cachexia and anorexia, metabolic alterations in malignancy and following anti-cancer therapies, and the role of nutrition, diet and dietary factors in the intervention, treatment and support of the patient with diagnosed cancer.

Accomplishments

Current studies are exploring various aspects of the relationship between nutrition and malignancy.

1. Heber and associates are continuing to examine the efficacy of enteral nutritional supplements in the prevention and treatment of protein-calorie malnutrition in the patient with lung cancer. Hormonal and metabolic aberrations will be examined in these patients to determine patterns of nutritional disease which may be amenable to specific nutritional intervention.

2. Bernstein, et al are continuing to conduct studies regarding the development of food aversions and taste alterations in children receiving cancer chemotherapies. The temporal relationship between the food items and the therapy, as well as the novelty of the food item, appear to be important in the development of food aversions.

3. Burholt, et al are examining the response of the gastrointestinal mucosa to radiation and various chemotherapeutic modalities in mice. Changes in food consumption pattern, gastric emptying, and the proliferative activity of intestinal epithelium are being studied as a function of dose and schedule of modalities known to be toxic to the gastro-intestinal mucosa. The response following different diets is also being examined.

2.33 Supportive Care (Under RO1 Grant Programs)

Description

This program activity was established to stimulate and support preclinical and clinical research activities aimed at the prevention and amelioration of side-effects resulting from therapeutic modalities or malignancy. This program activity includes new approaches to the management of pain, myelosuppression and its concomitant complications, which include protective environments, transfusion therapy, and infectious disease, as well as other preventive and supportive measures.

Accomplishments

A representation of the range of research activities supported is provided.

1. Alberts et al have continued the research initiated by Dr. Dorr at the University of Arizona looking into the skin toxicity resulting from extravasations of chemotherapeutic agents. Using a murine model, investigation continues into defining the lesions produced by different agents and improving the management of such lesions in the clinical setting.

2. Slichter, et al and Zaroulis, et al are investigating aspects of platelet alloimmunization in patients receiving prolonged transfusion therapy. Patients with acute luekemias and bone marrow transplants will be studied utilizing newer approaches, which include hybridoma technology.

2.34 Surgical Oncology

Description

This program was established to stimulate and support surgical oncology research efforts throughout the country and a Program Announcement for RO1 and PO1 grant applications was issued to encourage these efforts.

Accomplishments

At present there are 19 RO1 and 3 PO1 grants in the portfolio of the Surgery Section. In order to stimulate further activity in Surgical Oncology Research, the Director, DCT has authorized reissuing the RO1/PO1 program announcement four times this year.

2.4 PO1 Nutrition Grants

Two core grants which were currently supporting the development of Clinical Nutritional Research Units in major cancer centers have been transferred to DRCCA. These programs combine the resources of clinical and basic laboratories in geographically related hospitals and universities.

2.5 P20 Surgical Oncology Grants

An RFA for Surgical Oncology Planning Grants (P20) was published in 1981, and 28 applications were received in response. Of these, five were funded. A second P20 RFA may be reissued later in order to further stimulate planning for surgical oncology research.

2.6 Cooperative Agreements

2.61 Conversion of Cooperative Agreements

The GITSG, LCSG, and NSABP contracts were converted to the cooperative agreement funding mechanism. The Cancer Clinical Investigation Review Committee (CCIRC) plans to review the national multidisease groups and pediatric intergroups; the Cancer Regional Studies Review Committee (CRSRC) will review regional and site-specific groups.

Under the direction of the Program Director of the Clinical Cooperative Group Program each cooperative group submitted guidelines for quality control in the Fall of 1981. Every cooperative group has fully implemented a Quality Assurance Program and revised their guidelines with assistance of NCI/OPRR staff. Specific site visit dates and reports of visits have been submitted for NCI review and assistance. As monitoring programs develop the staff plans to become more involved. Program directors are submitting an annual report describing NCI involvement in each cooperative agreement.

2.62 Immunodeficiency Syndrome (AIDS)

Over the past year staff of the CIB have been heavily involved in NCI activities related to AIDS. Dr. Killen has chaired the NCI committee which developed an RFA for support of epidemiologic, laboratory, and clinical studies of this disease. The committee is composed of program staff from the NCI's Division of Cancer Biology and Diagnosis, Division of Cancer Cause and Prevention and Division of Cancer Treatment. In addition the committee assisted the Division of Research Grants, NIH, in expediting the initial review process of the applications received, made funding recommendations, and is now actively involved in the implementation of the Working Group. The NIAID has joined the NCI in funding of activities under the RFA. A total of 12 institutions are funded as of this writing (4 NIAID, 8 NCI) at an estimated annual total cost of over $3,000,000 per year. The projects funded span a spectrum of disciplines and involve widely dispersed geographic locations including major patient centers on both coasts. Major areas under study include the following:

Virology: Investigators at several institutions are involved in attempts to identify a viral agent. Methods include classical

isolation procedures, immunologic detection techniques, and nucleic acid hybridization.

Immunology: Research in this area involves further characterization of the nature of the immunologic defect in AIDS patients as well as patients with possible prodromes or in high-risk groups. Activity includes evaluation of all components of the immune response both in vivo and in vitro and at all levels, down to individual cell function. Also, under study are various antibodies, including ones directed at sperm and T-cells, and immune complexes or other evidence implicating a possible autoimmune etiology. Several investigators are also involved in immunogenetic profile studies, with preliminary evidence from several centers pointing toward a predominance of certain HLA haplotypes in affected patients.

Epidemiology: A number of affected AIDS or high-risk groups are under active prospective observation, including cohorts of male homosexuals, heterosexual drug addicts, native Haitians, children, and individuals with several possible prodromes, including the syndrome of diffuse lymphadenopathy. Plans for other studies are under development, and include multi-institutional investigators of health care workers, prisoners, etc.

Therapy: Included in this class are laboratory studies and therapeutic trials of prophylactic biologic response modifiers (BRM) for patients with opportunistic infections or high-risk prodromes; BRM, cytotoxic chemotherapy or irradiation for patients with Kaposi's sarcoma; and treatment studies for several of the common opportunistic infections, including Pneumocystis carinii and Cryptosporidiosis.

3.0 Contract Programs

3.1 Medicine Section

3.11 Instituto Nazionale por le Studio e la Cura dei Tumori

A major effort in breast cancer has been through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have received world-wide attention. Studies testing the value of noncross resistant drug regimens in the adjuvant setting are currently in progress, as are evaluations of adjuvant therapy in women with negative axillary nodes.

3.12 The International Bone Marrow Transplant Registry (IBMTR)

IBMTR is located at the Mount Sinai Medical Center in Milwaukee, Wisconsin. The Center is supported by a contract jointly funded

by the NCI and the NIAID, and is the largest source of data on transplantation in this country. Recent studies have concentrated on multifactorial analyses of prognostic factors and complications associated with the procedure.

3.13 Phase II-III Drug Evaluation Contracts

This contract is for Phase II-III studies to detect useful therapeutic effects of new drugs alone and in various combinations in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, ovary, endometrium, cervix, head and neck, stomach, pancreas and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. At each of the 4 involved institutions a minimum of 175 patients a year are studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery.

3.14 Immunotherapy

The Mount Sinai study in "Chemoimmunotherapy of Acute Myelocytic Leukemia" is showing a survival advantage for patients treated with chemotherapy plus allogeneic neuraminidase-treated myeloblasts over chemotherapy alone. Additional patient accrual is needed to obtain statistical significance.

The Yale study of "Intratumoral BCG Immunotherapy Prior to Surgery for Carcinoma of the Lung" has been phased out.

The Albany Medical College study "Intrapleural BCG After Primary Surgery for Lung Cancer" is treating Stage I patients with resectable disease. The BCG arm appears to be showing a negative correlation with survival and the study is currently being phased out.

3.2 Nutrition Section

Currently five contracts, engaged in a multi-institutional trial studying the effects of aggressive hyperalimentation in small cell lung cancer patients, are nearing completion. In a large, prospectively randomized trial which accrued over a hundred patients, hematologic recovery following chemotherapy was found to be accelerated in the patient population randomized to receive parenteral nutritional support. Several manuscripts are in preparation at this time. In another contract, Emory University has accrued patients with various malignancies and non-malignant conditions to evaluate the relationship between diet, activity, and response to therapy by means of direct and indirect calorimetry. Standardization with an aged-matched control populations is also being established.

Three additional contracts, developed to conduct a multi-instituitional trial examining the relationships between various new and previously accepted nutritional assessment parameters in patients with advanced colorectal and non-small cell lung cancers have now been convered to cooperative agreements. The three principal investigators have recently formed the Nutrition Oncology Research Cooperative Agreement (NORCA), a group which plans to continue pursuit of many of the aforementioned research areas in a multi-institutional approach.

3.3 Surgical Section

Head and Neck Contracts Program:

This is a collaboration of six institutions and two cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy.

The participating institutions are University of South Florida, University of Texas-Galveston, University of Cincinnati, University of Maryland, Memorial Sloan-Kettering Hospital, University of Michigan, Radiation Therapy Oncology Group, and Northern California Oncology Group. This study was initiated in October, 1978 and patient accession was discontinued in April, 1982 with a total accrual of 462 patients. Currently 276 patients are still alive and being followed. As this program concludes plans are underway to develop another head and neck cancer research group funded through the cooperative group or intergroup mechanism.

4.0 Miscellaneous

4.1 Intergroup Testicular Study

This is a collaboration between seven cooperative groups, including the CALGB which joined this fiscal year, and four large institutions having an interest in testicular cancer. The protocol is a randomized controlled study of adjuvant chemotherapy of Stage II resectable testicular cancer and a monitoring of Stage I testicular cancer. For Stage II the study compares the disease-free and overall survival for surgery alone (with combination chemotherapy for relapse) vs surgery plus early adjuvant chemotherapy. Stage I patients are registered and monitored to identify prognostic variables which may predict recurrence in this group. The protocol also includes important biologic studies such as histologic typing, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography. Progress presentations have been made at various cooperative group meetings: CALGB, SEG, SWOG, and NCOG.

Accrual statistics include:

Stage	4/14/80	5/12/81	6/1/82	5/31/83	
I	18	79	156	193	
II	37	100	148	181	(91 adjuvant; 90 adj)
total:	55	179	304	374	

4.2 Extramural Clinical Trials Office (ECTO) - EMMES

This contract provides administrative support to contract funded clinical research projects: LCSG, GITSG, H&N Project, Intergroup Testicular Study. The services provided include: assistance in protocol and forms design; patient randomization; quality control of data; coordination of scientific activities of clinical investigators, statisticians, and project officers; planning of meetings and preparation of agenda, minutes, reports, communications, and related administrative tasks.

4.3 NCI-Pan American Health Organization: Collaborative Cancer Treatment Research Program (PAHO:CCTRP)

The collaboration between US-Latin American investigators in the development of clinical studies continues to be the major goal of this program. An extensive re-evaluation of these goals has been done resulting in: 1) concentration primarily in Phase II trials in diseases prevalent in the area such as penile, vulvar, esophageal, head and neck, gastric, lung, and cervical cancer; 2) design of master Phase II protocols in the diseases listed above of which two have been approved and activated, and the remainder have been completed and will be submitted for activation; 3) the criteria of an internal scientific advisory committee to coordinate scientific directions, review and provide advice for protocols and publications, review and evaluate membership status; 4) careful assessment of feasibility and scientific merits prior to activation of new Phase III trials. These will preferably be performed by more than one center to ensure adequate accrual. More efforts will be made to assure proper patient entry, interim evaluation, reporting procedures and final analyses. These trials will be performed in diseases prevalent in this area of the world which should enable the groups to make unique contributions.

Two studies in Hodgkin's and non-Hodgkin's lymphomas have been closed and generated interesting data. A Phase III trial comparing chemotherapy alone vs chemo-radiation therapy in patients with all stages of Hodgkin's disease continues to accrue patients and within one year should provide us with important information. A pilot study with intra-arterial platinum in osteosarcoma has been completed and will provide us with the information and experience needed for the design of the planned Phase III adjuvant study in this disease.

5.0 Staff Publications

deMoss, E.V., Lichter, A.S., Lippman, M., Gerber, N.L., Reichert, C.M., Edwards, B.K., Schain, W.S., Gorrell, C.R., D'Angelo, T., and Rosenberg, S.A.: Complete axillary lymph node dissection prior to radiotherapy for primary breast cancer. Alternatives to Mastectomy. Proceedings of the Symposium. J.B. Lippincott. (In press).

DeWys, W.D., Killen, J.Y.,: The paraneoplastic syndromes. Rubin, P. (Eds): Clinical Oncology for Medical Students and Physicians: A Multidisciplinary Approach. 6th Edition. New York, American Cancer Society. (In press).

Edwards, E.K., Jacobs, E.M. (Chairmen), Edwards, B.K., (Ed.): Role of Computers in in Cancer Clinical Trials, DHHS, NIH, NCI, CTEP, CIB, May 1983, pp. 1-188. (In press).

Eisenberger, M.A., et al.: Combination chemotherapy with bleomycin and hydroxyurea in the treatment of advanced head and neck cancer. Cancer Treatment Reports. 66: 1439-1440, June 1982.

Killen, J.Y., Hoth, D.F., Smith, F.P., Schein, P.S., Woolley, P.V.,: Phase II Studies of methyl-glyoxal-bis-guanylhydrazone in carcinoma of the colon and lung, Cancer, 50 (7): 1258-1261, 1982.

Killen, J.Y., Ellenberg, S.S., Adjuvant chemotherapy and immunotherapy of gastrointestinal cancer. In Levin, B. and Riddel, R. (Eds.):, Current Concepts in Oncology: Gastrointestinal Cancer. New York, Elsevier North-Holland. (In press).

Lichter, A.S., Lippman, M.E., Gorrell, C.R., D'Angelo, T., Edwards, B. and deMoss, E.V.: Adjuvant chemotherapy in patients treated primarily with irradiation for localized breast cancer. Alternatives to Mastectomy. Proceedings of the Symposium. J.B. Lippincott, 1982 (In press).

Lippman, M.E., Lichter, A.S., Edwards, B.K., Gorrell, C.R., D'Angleo, T., and deMoss, E.V.: The impact of primary irradiation treatment of localized breast cancer on the ability to administer systemic adjuvant chemotherapy. J. Clin. Oncol, 1982. (In press)

Muggia, F.M., DeWys, W.D.: Staging in testicular teratomas. In White-house, J.M.A. and Williams, C.J. (Eds): Recent Advances in Clinical Oncology, London, Churchill - Livingston, London, 1982, pp. 85-94.

Rosenberg, S.A., Tepper, J., Glatstein, E., Costa, J., Young, R., Baker, A., Brennan, M.F., deMoss, E.V., Seipp, C., Sindelar, W.F., Sugarbaker, P. and Wesley, R.: Prospective randomized evaluation of adjuvant chemotherapy in adults with soft tissue sarcomas of the extremities. Annals of Surgery 196: 305-315, 1982.

Weiss, R.B., DeWys, W.D., Green, S.B., Williams, S.D., Einhorn, L.R., Muggia, F.M., Golbey, R.B., Brunner, K.W., and Jacobs, E.M.: Advuvant Chemotherapy and Indicators of Relapse in Stages I, II Non-Seminomatous Testicular Carcinoma. Proceedings 13th Int Congress of Chemotherapy, Vienna, Austria, 1983 (In press).

6.0 Staff Presentations

Ernest deMoss, M.D.

1. Surgical Alternatives in Breast Cancer - Treatments for Primary Breast Cancer Other Than Radical Mastectomy. Arlington Cancer Treatment Center, Arlington, Texas, January 21-22, 1983.

Edwin Jacobs, M.D.

1. Important Phase III/IV Trials in the U.S. in Gastrointestinal Cancer, U.S. - Japan Eight Annual Treatment Program Area Review Meeting, National Cancer Institute, Bethesda, Maryland, November 22-23, 1982.

2. Recent Analysis of Data for Intergroup Testicular Protocol, Semiannual Meeting of SWOG, March 11, 1983

3. Recent Developments in Intergroup Testicular Protocol, Semiannual Meeting of NCOG, April 29, 1983

4. Recent Developments in the Intergroup Testicular Protocol, Semiannual Meeting of CALGB, May 5, 1983

Jack Killen, M.D.

1. Recent Developments in Gastrointestinal Cancer Therapy. Annual Meeting of the Piedmont Oncology Association. Asheville, North Carolina, October 14-15, 1982.

2. Recent Developments in Breast Cancer Therapy and Recent Developments in Gastrointestinal Cancer Therapy. Meeting of Representatives of the Italian - American Agreement on Treatment of Cancer. Palermo, Sicily, October 14-15, 1982.

3. Important Phase III/IV Trials in the U.S. in Gastrointestinal Cancer. US/Japan Eight Annual Treatment Program Area Review Meeting, National Cancer Institute, Bethesda, Maryland, November 22-23, 1982.

Richard Ungerleider, M.D.

1. Agents for Phase II Studies in Children With Cancer. Childrens Cancer Study Group Meeting, Chicago, Illinois, October 11, 1982; CCSG Meeting, Salt Lake City, Utah, February 10, 1983.

2. Agents for Phase II Studies in Children With Cancer. Pediatric Oncology Group Meeting, St. Louis, Missouri, October 4, 1982.

7.0 Conferences - Workshops - Seminars

1. Executive Committee and Panels of the National Testicular Group, October 14, 1982.

2. Working Group Meeting on Pilot Studies Using Radiolabeled Antiferritin, November 18, 1982.

3. Conference on Role of Surgery in Limited Small Cell Lung Cancer, November 19, 1982.

4. Workshop on the Role of Surgery in Lung Cancer, December 2, 1982.

5. Conference of the Executive Group Chairmen, December 3, 1982.

6. Working Meeting with Cooperative Agreement - Development of Nutrition in Clinical Trials, December 6, 1982.

7. Future Directions in Clinical Trials - Head and Neck Cancer, January 13-14, 1983. (Salt Lake City, Utah).

8. Workshop on Intergroup Studies in Pancreatic Cancer, February 4, 1983.

9. Future Directions: Cooperative Agreement - Nutrition in Clinical Trials, February 15, 1983.

10. Workshop on Side Effects of MeCCNU in Clinical Trials, February 22, 1983.

11. Cooperative Group Chairmen Semiannual Meeting, March 21, 1983.

12. Clinical Trials in Prostate Cancer - NPCP, April 5, 1983.

13. Clinical Treatment Trials of the PAHO - April 6, 1983.

14. Clinical Trials Working Group, NCI, CTEP, CIB, April 15, 1983.

15. Group Discussion - Cooperative Agreement - Nutrition in Clinical Trials, April 17, 1983.

16. Conference on Bone Marrow Transplantation, April 26, 1983.

17. Meeting of Executive Committee - National Testicular Study, April 28, 1983.

18. Meeting - AIDS Working Group, May 6, 1983.

19 Workshop for Computers In Clinical Trials, July 27, 1983.

20. Working Group Meeting - Intergroup Study in Head and Neck Cancer, May 18, 1983.

INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch has the mission of sponsoring new investigational drugs for clinical trials and of evaluating them for antitumor efficacy. It does this by pursuing several objectives: (1) obtaining Investigational New Drug exemption (IND) authorization from the Food and Drug Administration (FDA), (2) managing and monitoring Phase I trials of new agents developed by the DCT, (3) planning with the Clinical Investigations Branch of CTEP Phase II trials in specific tumor types and subsequently monitoring the results of the clinical trials, (4) meeting FDA regulatory requirements for all active INDs, (5) regulating the distribution of investigational new drugs, and (6) maintaining close contact with the pharmaceutical industry participating in the development of new investigational drugs.

The Investigational Drug Branch is now divided in five sections. Two medical sections, one for the cytotoxic agents and one for the biologic response modifiers are concerned with the clinical aspects of the drug development process; a Drug Regulatory Affairs Section provides a constructive interaction with the Food and Drug Administration; the Drug Management Section regulates the distribution of investigational new drugs to all NCI sponsored investigators, and finally a Quality Assurance and Compliance Section has the mission of monitoring all investigators performing clinical trials with NCI sponsored agents in order to assure data quality and investigator compliance to FDA and HHS regulations. The professional staff of the Branch includes eight physicians, four Ph.Ds. and two pharmacists.

During the past year monitoring of some 1300 protocols for more than 90 INDs continues. Five new drugs entered Phase I testing, and Phase II trials began on 5 drugs.

The major administrative intiatives during this year included:

(1) Monitoring of investigational drug trials. A formal policy for site visit monitoring of investigational drug trials, approved by the DCT Board of Scientific Counsellors in June 1982, was fully implemented during this year. This will include a tri-annual site visit to each primary institution. In addition, the site visits will also be conducted at all affiliates participating in NCI supported IND drug studies. Plans were developed to monitor additional clinical trials sponsored through the Community Clinical Oncology Programs and the clinical trials in the organ site program. All major cooperative groups have embarked on on a program of site visit monitoring. More than 200 site visits were conducted during the current year. During the last quarter of the year the site visit program was expanded to include the cancer centers. A professional staff was recruited. Numerous meetings were held with investigators to inform and educate them of these new policies. (See below for additional details).

(2) Adverse drug reactions. Approximately 182 adverse drug reactions were evaluated and communicated to the Food and Drug Administration. A further clarification of the NCI policies on adverse drug reactions was developed and mailed to all investigators in January 1983.

(3) Informed consent. A formal system of informed consent review was established during this year. All informed consents are checked to assure their inclusion of all elements required by regulation.

(4) Drug accountability. A instruction manual was mailed to more than 5000 investigators in February 1983. This manual implemented the DCT policy on accountability for investigational drugs. All use of investigational drugs will now be recorded on a patient by patient basis.

(5) Pharmacokinetic Working Group. A pharmacokinetic working group was established within the Phase I Working Group this will bring together scientists who are interested in clinical pharmacokinetics.

(6) Toxicity Criteria. Common toxicity criteria were adopted by the Phase I Working Group.

(7) CTEP Letter. The CTEP Letter is now regularly mailed to a selected group of investigators (Cooperative Group Chairmen, Phase I/II contractors). The Letter has been designed to convey to the investigators to status of Phase I and Phase II drugs within the NCI clinical trials mechanism. It also suggests to the investigators what trials are needed to complete a clinical profile on each drug. A section of the Letter also includes reports of recent adverse reaction and any other information pertaining to the clinical trials ongoing under CTEP sponsorship. Sixteen drugs have appeared in the CTEP Letter.

DRUG EVALUATION AND REPORTING SECTION

Phase I Studies. Five investigational drugs sponsored by the DCT were newly introduced into Phase I clinical trials.

2-Fluoro-Ara-AMP	NSC-312887
Tiazofurin	NSC-286193
Spiromustine	NSC-172112
Acodazole	NSC-305884
SR-2508	NSC-301467

2-Fluoro-Ara-AMP is a halogenated analog of Ara-A which is not deaminated by adenosine deaminase. It has shown good activity in P388, L1210, and in the LX1. Five Phase I trials started utilizing a daily X 5 continuous infusion or bolus administration, single, and q12 hr X 3 day schedule. The starting dose was 1/10 of the mouse equivalent LD10. Biologic activity was observed at the starting dose in the single schedule trial. As a consequence all other trials were deescalated and we are presently in the process of determining a Phase II trial dose. The dose limiting toxicity is myelosuppression. In two patients signs of CNS toxicity were observed.

Tiazofurin. Tiazofurin is an antimetabolite with an unusual structure which appears to inhibit DNA synthesis by inducing a state of guanine starvation. Tiazofurin has striking activity in one of the most resistant of animal tumors, Lewis lung carcinoma, (out of 558 drugs tested in this system only 2.3% were active). The activity was maintained over a three log difference in doses. Tiazofurin is also active in L1210 and P388. The starting dose of the clinical trials was 1/10 of the mouse equivalent in the dog. Five Phase I studies have been initiated in the adult population utilizing daily X 5, daily X 3, and single bolus administration. Because of the block of IMP dehydrogenase induced by this compound and the consequent accumulation of IMP we expected to observe hyper uricemia in humans even at low doses. In fact hyperuricemia did manifest at the starting dose and all patients are now treated with allopurinol.

Spiromustine. Spiromustine is a compound which has been specifically designed for CNS tumors. It consists of a mustard moiety attached to a hydantoine radical ecule. Spiromustine has DN2 activity in the B16 carcinoma, Colon 26, IP-IC ependmyoblastoma, P388 leukemia, and the subrenal capsule mammary xenograft. Moderate activity was also found in the murine mammary in the colon 38 and the L1210 leukemia. Five Phase I studies utilizing a daily X 5, daily X 3, and bolus schedules has been recently started. No data are available so far.

In addition to the Phase I studies just mentioned, Phase I trials were continued from last year on the following drugs:

CBDCA
Dihydro-Azacytidine
Homoharringtonine
N-methylformamide
Tricyclic nucleoside
Echinomycin
Misonidazole + Cytoxan
Ara-A & DCF
Bisantrene 72 hr. infusion

Four further drugs are expected to enter Phase I trials before the end of the year: Caracemide, Menogarol, Taxol, and Rapamycin.

Several Phase II clinical trials have been conducted under the sponsorship of NCI and presented regularly at the Phase II meetings for the following drugs:

Alacinomycin
AZQ
Mitoxantrone
Bisantrene
PCNU
Methyl G
Alanosine
Deoxycoformycin
AT-125
Spirogermanium
Dichloromethotrexate

Phase III trials have been initiated for Mitoxantrone and Bisantrene in breast cancer.

A plan for the evaluation of two platinum analogs, CBDCA and CHIP, has been devised by the NCI in collaboration with Bristol and is now under implementation. Randomized, controlled, sequentially designed, Phase III trials comparing CBDCA to CHIP have been organized for head and neck, cervix, and bladder carcinoma in order to explore the spectrum of activity of the two analogs in platinum-sensitive diseases, and, at the same time, to retrieve information on the toxicity difference between the two analogs. Straight Phase II trials have been designed for diseases insensitive to platinum (i.e., colon, breast, etc.) to explore the possibility of a difference in spectrum of activity of those compounds. Phase II studies in ovarian carcinoma have also been initiated. In selected patients who cannot received platinum for medical reasons CBDCA and CHIP will be given as first line therapy.

Activity has been confirmed for the following drugs in respective diseases:

Mitoxantrone	Breast (confirming earlier results) leukemias, lymphomas, hepatomas
AZQ	Primary and secondary brain tumors lymphomas
Bisantrene	Breast
Spirogermanium	Lymphomas
2'Deoxycoformycin	T-cell lymphomas and leukemias, CLL
Dichloromethotrexate	Bladder, Cervix, and Head and neck

Biochemical Modulator Advisory Group (BMAG). The BMAG has been reorganized and new members have been appointed (see attached list). At the March meeting a workshop on the modulation of alkylating agents was organized. A workshop of intercalating agents is scheduled for the June meeting.

Pharmacokinetic. A pharmacokinetic subcommittee of Phase I Working Group has been created. Dr. Louis Malspeis of Ohio State University has been appointed as the Chairman of the Committee. The scope of this committee is 1) to organize and streamline the pharmacokinetic studies that are conducted under the Phase I/II trials, 2) to constitute the extramural arm of the Blood Level Working Group, and 3) to organize pharmacokinetics presentations at the Phase I/II Meetings.

Other Activities.

A meeting on Deoxycoformycin has been held in February 1983 and the proceedings of the meeting will be published in Cancer Treatment Reports.

The following workshop will be organized this summer.

1) Mitoxantrone cardiotoxicity (in conjunction with the Phase II meeting in July.

2) Workshop on new ways of giving old drugs: Adriamcyin will be held jointly with ADRIA lab in September. The experimental and clinical data on the continuous infusion, weekly schedule of Adriamycin will be presented and discussed.

DRUG REGULATORY AFFAIRS SECTION

IND SUBMISSIONS

For the fiscal year 1983, a Notice of Claimed Investigational Exemption for a New Drug (IND) was submitted to the Office of Drugs, Food and Drug Administration (FDA) for each of the following seven compounds:

Drug	NSC No.
Fludarabine Phosphate	NSC-312887
SR-2508	NSC-301467
Tiazofurin	NSC-286193
Acodazole	NSC-305884
Spiromustine	NSC-172112
Caracemide	NSC-253272
Menogarol	NSC-269148

The IND for the following drug was discontinued due to a lack of clinical activity:

Anguidine	NSC-141537

The Drug Regulatory Affairs Section also supported IND-related activities for the biological response modifiers. This support included preparation, submissions, and maintenance of INDs and support and monitoring of drug distribution.

The following IND's were submitted to the Office of Biologics, FDA, for the biological response modifiers:

Interferon (Human Fibroblast)	NSC - not yet assigned
Monoclonal Antibody (9.2.27) (IgG_{2a} Human Melanoma Antigen, Murine)	NSC-361437
Interleukin 2 (Jurkat)	NSC - not yet assigned
Radiolabeled Monoclonal Antibreast Cancer Antibody (Murine) (Whole and Fragments)	NSC - not yet assigned

DISTRIBUTION OF DELTA-9-TETRAHYDROCANNABINOL) (THC)

With the approvals of both the Oncology Advisory Committee and the FDA, Delta-9-Tetrahydrocannabinol (THC, NSC-134454), was placed under the NCI's Group C Distribution Mechanism for use as an antiemetic in cancer chemotherapy. Guidelines for THC use and a procedures document for distribution were developed. Since THC is also a Schedule I drug, concurrence on the distribution procedures was obtained from from the Drug Enforcement Administration (DEA).

THC is distributed only to hospital pharmacies which met IDB standards and are specifically registered for THC distribution. Presently there are 720 hospital pharmacies registered (about 10% of all hospital accrediated by the Joint Commission on Accrediation of Hospitals) to dispense THC for 2,602 approved physicians. More than one million capsules have been dispensed to approximately 25,000 patients. Distribution of THC as an antiemetic under state and individual investigator studies is also an IDB function. Currently, there are eleven state controlled studies and ten individual investigator studies in active status.

NEW DRUG STUDIES

There are presently forty-four Cancer Centers and fourteen New Drug Studies Groups participating in the New Drug Studies Mechanism. This represents a slight increase over last year. These institutions submitted ninety-eight protocols for the fiscal year 1983. Currently there are 265 active protocols.

ADVERSE DRUG REACTION

As outlined above approximately 182 adverse drug reactions (ADR's) were evaluated and reported to the FDA. The ADR's were evaluated by the Division of Cancer Treatment's Adverse Drug Reaction Committee (ADRC) which is made up of staff from both the Developmental Therapeutics Program and the Cancer Therapy Evaluation Program. The Committee meets monthly to discuss the ADR's submitted the previous month to FDA. The ADRC determined any further actions which are necessary, e.g., amending the Informed Consent Form and the Clinical Brochure and warning letters to investigators. Final resolution letters are submitted to the FDA after the ADRC has discussed and evaluated an ADR. The final resolution includes a key which lists all information concerning that ADR from the time it is reported to IDB until it is evaluated by the ADRC and includes any subsequent actions suggested by the ADRC.

QUALITY ASSURANCE AND COMPLIANCE SECTION

The NCI Site-Visit monitoring program was initiated in June 1982 upon approval of the DCT Board of Scientific Counselors. All fourteen cooperative group participating in investigational drug trials have submitted their plans for site-visiting their full members. All fourteen are actively implementing their program:

Cancer and Leukemia Group B
Children's Cancer Study Group
Eastern Cooperative Oncology Group
North Central Cancer Treatment Group
Pediatric Oncology Group
Gynecologic Oncology Group
Northern California Oncology Group
Radiation Therapy Oncology Group
National Surgical Adjuvant Breast Group
Southeastern Group
Southwest Oncology Group
Mid-Atlantic Oncology Program
Piedmont Oncology Association
Gastro-Intestinal Treatment Study Group

A total of 125 group members have been audited to date roughly representing 1/3 of the group membership. In this fiscal year, monitoring is also being accelerated to include all affiliate members over a 12-18 month period. Approximately 65 members have been audited to date. Quality Assurance and Compliance Section has co-site visited is about 20%. By the end of the fiscal year approximately 1/5 of the cancer centers would have been audited.

During this fiscal year, guidelines for affiliate membership, on-site auditing drug accountability, and audit reports were developed.

DRUG MANAGEMENT AND AUTHORIZATION SECTION

Drug Accountability. A drug accountability system has been designed, field tested, and implemented during the past year. This system was designed to account for each drug ordered for the protocol it is to be used for. All investigational drugs including Group A, B, C, and Special Exceptions are to be accounted for by this system. The investigator ordering the drug will be responsible for this accountability whether the drug is used in their institution or a satellite.

Single Investigator Address for Drug Shipments: During the past year all investigators have assigned a single drug shipping address rather than the multiple shipping address used in the past. Investigators are permitted to have a second address if their office address is different from the drug shipment address. This should increase the efficacy of the accountability system as well as provide a better list of investigator addresses for mailing adverse drug reaction, etc.

Drug Distribution and Computer Modifications: During the past fiscal year the following modifications have been made to the storage of material in the drug distribution computer system:

1) removed all terminated protocols from the files and this information has been stored on magnetic tapes,

2) removed from the inactive file all investigators with no activity since 1974 this was 3,788 investigators,

3) removed all terminated group codes from the investigator file,

4) updating investigator registration date once a year instead of several times a year as we have received materials from various cooperative groups, and

5) reduce the frequency of several reports.

The following are some of the drug distribution data for the past year.

Total Number of Drug Orders	Total Number of Line Items per Drug Request	Total Number of Line Items on Group C Drugs	Total Number of Line Items for Special Exceptions
13,784	22,550	6,446	2,112

STAFF PUBLICATIONS:

1. Bender, J. F., Grillo-Lopez, A.J., Posada, J.G., Jr.,: Diaziqunone (AZQ). Investigational New Drugs (IND), 1: 71-84, 1983.

2. Danhauer, F.L., Fortner, C.L., Schimpff, S.C., DeJongh, C.A., Wesley, M., Wiernik, P.H.: Ototoxicity and Pharmaceutically Determined Dosages of Amikacin in Granulocytopenic Cancer Patients . Clin. Pharm., 1: 539-543, 1982.

3. DeJongh, C.A., Wade, J.C., Schimpff, S.C., Newman, K.A., Finley, R.S., Salvatore, P.C., Moodey, M.R., Standiford, H.C., Fortner, C.L., and Weirnik, P.H.: Empiric Antibiotic Therapy for Suspected Infection of Granulopenic Cancer Patients - A Comparison Between the Combination of Moxalactam plus Amikacin and Ticarcillin plus Ammkacin. Am. J. Med., 73: 89-96, 1982.

4. Finley, R.S., Fortner, C.LK., DeJongh, C.A., Wade, J.C., Newman, K.A., Caplan, E., Britten, J., Wiernik, P.H., Schimpff, S.C.: Comparison of Standard Versus Pharmacokinetically Adjusted Amikacin Dosing in Granulocytopenic Cancer Patients, Antimicrob. Ag. Chemother. 22 (2): 193-197, 1982.

5. Finley, R.S., Schimpff, S.C. Fortner, C.L., and Weirnik, P.H.: Rifampin and Cloxacillin in the Reduction of Staphylococcus aureus Colonization. Clin. Pharm., 1: 370-372, 1982.

6. Fortner, C.L. Finley, R.S., and Schmipff, S.C.: Piperacillin Sodium: Antibacterial Spectrum, Pharmacokinetics, Clinical Efficacy and Adverse Reactions, Pharmacotherapy 2: 287-299, 1982.

7. Grove, W.R., Fortner, C.L., and Wiernik, P.H.: Review of Amsacrine, an Investigational Antineoplastic Agent. Clin. Pharm. 1: 320-326, 1982.

8. Hoth, D.F, Posada, J.G.: Clinical Trials of Aclacinomycin A in USA Sponsored by NCI. Proceedings of the 13th International Congress of Chemotherapy: Vienna, Austria, August 28-30, 1983.

9. Lev, L.M. and Posada, J.G.: Aclacinomycin Status of Phase I and II Studies in the United States. Cancer Treatment Reports. In press

SUMMARY REPORT
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
OCTOBER 1, 1982 - SEPTEMBER 30, 1983

I. Introduction

The Radiation Research Program was formally approved by the Secretary for Health and Human Services on May 17, 1982. The proposal to establish the Radiation Research Program within the Division of Cancer Treatment was based on recognition of the potential scientific advantages of coordinating radiation research activities and the need to provide an organizational context which would emphasize and encourage a more coherent approach to research in these areas. The three Branches which comprise the Radiation Research Program are the Diagnostic Imaging Research Branch, the Low-Level Radiation Effects Branch, and the Radiotherapy Development Branch.

The mission of the Radiation Research Program is the planning, development, administration, and evaluation of an extramural radiation research program through establishing program priorities, allocation of resources, maintaining project integration, evaluation of program effectiveness, and representing the program area in the management and scientific decision making processes of the National Cancer Institute. This requires the coordination of research program activities with related programs elsewhere at NCI and NIH, with other Federal agencies, and with national and international research organizations. The RRP also provides a focal point within NIH for extramural investigators nationally and internationally on radiation research.

II. Personnel

A. Staffing

1. Office of the Associate Director

David Pistenmaa, M.D., Associate Director
Francis Mahoney, Acting Deputy Associate Director
Oddvar Nygaard, Ph.D., Special Assistant for Low-Level Radiation
Glenn Sheline, M.D., Special Assistant for Radiotherapy
Gabriel Wilson, M.D., Special Assistant for Diagnostic Imaging
Bonnie Jenkins, Secretary to Associate Director
Elizabeth Swerda, Secretary to Special Assistant for Radiotherapy
Jan Johnson, Technical Assistant
Maureen Volz, Statistical Assistant

2. Administrative Office

Dorothy Tisevich, Administrative Officer
Louise Patten, Budget Assistant
Pamela Keys, Summer Student

3. Diagnostic Imaging Research Branch

David Pistenmaa, M.D., Acting Chief
Matti Al-Aish, Ph.D., Program Director for X-Ray Imaging
Roger Powell, Program Director for Diagnostic Imaging
Bernice Nasoff, Branch Secretary

4. Low-Level Radiation Effects Branch

Bruce Wachholz, Ph.D., Chief
James Murray, D.V.M., Program Director
Ann Malner, Branch Secretary

5. Radiotherapy Development Branch

Alfred Smith, Ph.D., Acting Chief
George Alexander, M.D., Cancer Expert
Richard Cumberlin, M.D., Cancer Expert
Thomas Strike, Ph.D., Chief, Radiation Modifier Section
Anne Gentilcore, Branch Secretary

B. Recruitments

Robert Burt, M.D., Special Assistant for Diagnostic Imaging
Chief, Diagnostic Imaging Research Branch
Clerk-Typist, Radiotherapy Development Branch

III. Major Activities

The major activities of the RRP have been devoted to organizing radiation activities in DCT and NCI and to developing new initiatives for the Radiation Research Program. To further these goals long-range plans have been developed for diagnostic imaging and for radiation therapy.

A long-range plan for diagnostic imaging research was developed under the direction of Dr. Gabriel Wilson, Chairman, Department of Radiology, University of California, Los Angeles, during his year with the Radiation Research Program. Dr. Wilson worked with the Conjoint Committee for Radiology which consists of members of the American College of Radiology, the Association of University Radiologists, and the Society of Chairmen of Academic Radiology Departments and is chaired by Dr. James Youker of the Medical College of Wisconsin. This plan will be published in Investigative Radiology in late 1983 or early 1984.

A long-range plan for radiation oncology, biology, and physics was developed under the direction of Dr. Glenn Sheline, Vice-Chairman of Radiology, University of California, San Francisco, during his year with the Radiation Research Program. Dr. Sheline worked with the Commission on Radiation Therapy of the American College of Radiology chaired by Dr. Luther Brady of Hahnemann Medical College. The plan will be published in Cancer Treatment Reports in late 1983. This plan also contains material pertinent to the goals and objectives of the Low-Level Radiation Effects Branch.

IV. Radiation Research Program Research Grant and Contract Support

FY83 BUDGET
(Dollars in Thousands)

	Contracts Number	Contracts Amount	Grants Number	Grants Amount
Diagnostic Imaging Research Branch	1	---	66	$ 9,181
Low-Level Radiation Effects Branch	8	$2,971	49	$ 5,563
Radiotherapy Development Branch	24	$5,694	167	$37,451
TOTAL RRP	33	$8,665	282	$52,195

V. Scientific Overview

The RRP is concerned with support of research into the medical uses, as well as the adverse effects, of both ionizing and non-ionizing radiations. Being a new Program, the scope of research supported in some areas, especially in diagnostic imaging and in low-level radiation effects, is limited. Research in diagnostic imaging is supported by other Institutes with interests in specific applications of imaging whereas low-level radiation effects research is generously supported (relatively) by the Department of Energy and to a lesser extent by several other government agencies. Research in radiation oncology, biology, and physics receives a large share of the RRP resources because of its existence as an activity at NIH for over a decade. Summaries of the research supported by each of the three Branches are presented in Attachments 1-3.

Several exciting areas of research are supported by the Diagnostic Imaging Research Branch. Just as new developments in CT scanning are declining the field is stimulated by nuclear magnetic resonance (NMR) imaging, labeled monoclonal antibody imaging, and photoelectronic (digital subtraction) imaging. NMR promises to provide significant information on physiological function or pathophysiologic states as well as superb anatomical images in any body plane of interest without the use of ionizing radiations. The most important area of research is the study of the relationships among the basic parameters of NMR imaging (T_1 and T_2 relaxation times) and both normal and pathological states of tissues. The Branch has received proposals for a contract-supported research effort to evaluate NMR alone and to compare it to other imaging modalities in patients with selected diseases, including cancer. In addition, the Branch is issuing a request for applications for grants to study the basic parameters of NMR imaging. Each of these initiatives is budgeted at a level of $1,000,000 per year for three years. The development of monoclonal antibodies to specific diseased tissues, especially cancers, may greatly improve the detection and diagnosis of selected diseases. In addition to supporting research on methods of labeling antibodies with isotopes appropriate for external imaging, the Branch supports research to develop single photon emission computed tomographic (SPECT) imaging systems which are both complementary to and essential for the optimal exploitation of the use of labeled antibodies for imaging. Photoelectronic imaging is a maturing area of research in that it is moving from the early development phase into relatively wide clinical evaluation. This digital subtraction technique, which uses intravenously injected contrast media, provides images comparable to those obtained with intra-arterially injected contrast agents. Other related research areas include the development of new radio-

active isotopes for use in nuclear medicine; the development of new contrast agents for x-ray, ultrasound, and NMR imaging; and the study of the potential bioeffects of ultrasound.

The Low-Level Radiation Effects Branch is supporting a number of important research projects through contracts as well as grants in an attempt to understand the molecular and cellular interactions of radiation which lead to mutagenesis, cell transformation, and carcinogenesis, especially at low radiation doses or low dose rates. In the area of human radiobiology, an epidemiological study was initiated in FY82 to provide an assessment of leukemia and thyroid disease in relation to fallout in Utah. The Department of Energy and the Department of Defense participate in the funding of this contract. Two other followup studies are designed to estimate the risk of human thyroid cancer from radiation exposures. One is a study of patients who had iodine 131 and other diagnostic procedures during childhood and adolescence, while the other is a continuing study of patients with a history of external irradiation to the head and neck during childhood.

Several studies supported by the LLRE Branch concern the induction of malignancies in mice by ionizing radiation. In one of these, the experimental protocol is to irradiate different strains of mice with single doses, repeated doses, and with a wide range of dose rates. In another study, the induction of radiation-induced myelogenous leukemia by fission neutrons is being observed in mice to establish a reliable dose-response function as well as to relate induction of malignancy to the production of chromosome aberrations. Two additional studies in rodents involve induction of cancer by ionizing radiation in combination with a second agent.

Two projects support investigations of the variation in sensitivity of animals to ionizing radiation as a function of stage of development. One of these is an evaluation of the types and frequencies of neoplasms occurring in dogs that have been irradiated at various times during development. A study in rodents addresses primarily teratogenesis and other toxic effects on embryos but also will include an evaluation of the induction of leukemia in prenatally exposed mice. Unique differences between the effects of X-irradiation and various energies of neutron radiation have been demonstrated.

The effects of ionizing and ultraviolet radiation on cells are being studied in a wide variety of cell types from humans, Chinese hamsters, rats, mice, plants, and bacteria. Each cell type offers certain advantages for studying unresolved aspects of cell killing, malignant transformation, and mutation.

A team of investigators has developed a reproducible, quantitative assay for carcinogen-induced mutation or neoplastic transformation of diploid human fibroblasts. They plan to use these assay systems to determine the response of such cells to the transforming and mutagenic actions of low doses of ionizing radiation. They will also determine whether repair of sublethal or potentially lethal damage influences these effects of the radiation, or if cells from cancer prone individuals respond differently.

Other important areas of research include the following:

- o One research group is investigating the UV light-induced transformation of cultured human cells, the effects of UV on DNA damage and repair in cultured cells and in skin, and the relation of these areas to solar oncogenesis in man.

- The aim of another study is to gain further information on the nature of the radiation response of the cells and the number of sensitive cells involved in mammary carcinogenesis in mice.

- In one low-level radiation study, the dose-response curve has been established for stamen hair somatic mutation in the Tradescantia plant following exposure to ^{137}Cs gamma rays and has confirmed the hypothesis of linearity below about 7.5 rad.

- NCI is supporting one of the very few projects in the world which attempts to look at rapid kinetics of chemically induced changes in cellular radiosensitization. This project is concerned with obtaining some understanding of the early physicochemical events involved in radiation effects in cells. Another part of the program focuses on the identification of initial radiation damage to DNA, its repair, and factors that may influence this damage and repair at the chromosomal level. The primary emphasis is on ionizing radiations but some projects involving ultraviolet radiation are also included.

- The importance of the chromosomal structure and function in the sensitivity of specific regions of DNA is addressed in two projects.

- The involvement of DNA as a target in carcinogenesis is addressed in a study to test the hypothesis that enhanced DMBA tumorogenesis in a hamster cheek pouch model by X-radiation is due, wholly or in part, to the radiation induction of alterations in the binding of DMBA to the DNA of the target epithelial cells.

- Three other projects involve studies of the effects of UV radiation and the repair of UV-induced damage in DNA.

The Radiotherapy Development Branch is by far the largest component of the Radiation Research Program and administers a large grant- and contract-supported research program encompassing radiation oncology, biology, and physics. The largest areas of research in terms of funding are particle beam radiotherapy, hyperthermia, and general radiobiology. Support of research in the development of radiosensitizers and radioprotectors, in intraoperative radiotherapy, in photoradiotherapy, and in physics have also been important activities of the Branch in FY83.

The particle beam radiotherapy research activities are multifaceted and include strong radiobiology and physics, as well as clinical research components. The establishment of clinically dedicated neutron therapy facilities is being completed at the Fox Chase Cancer Center, M. D. Anderson Hospital, and the University of Washington while progress at the University of California, Los Angeles, has been delayed slightly by the bankruptcy of the manufacturer of the cyclotron. Encouraging preliminary results are being reported from the existing neutron therapy facilities in studies coordinated by RTOG on malignant gliomas, neck nodes, prostatic carcinoma, and bladder cancer. Very promising results have been observed at the Lawrence Berkeley Laboratory and the Harvard cyclotron in the treatment of posterior uveal melanomas with helium ions and protons, respectively. Treatment of other selected tumors at these facilities has convincingly demonstrated the dose localization advantages of proton and helium ion beams. The follow-up evaluation of patients treated with pions at Los Alamos continues even though treatment at that facility stopped in May 1982.

Interest in the potential use of hyperthermia as a potent modifier of the response of tumors to radiation and chemotherapy has led to an increasing number of grant-supported projects in this area of research. Investigations now supported include the study of basic effects of heat alone or in combination with radiation on DNA crosslinks, membranes, cells, tumors and their blood vessels, normal tissues and the immunological response; of the effects of blood flow, the cellular environment, the development of tolerance to heat, and of the duration and temporal relationship of the heating to the radiation; and of the development of methodology for heating (extracorporeal, interstitial, external) and for thermometry. The Branch is supporting by contract a Hyperthermia Working Group of five leading institutions in this country which is conducting an evaluation of equipment available for heating cancers and for measuring temperatures therein and is developing guidelines for the use of this equipment by other investigators. A contract has been awarded to establish a hyperthermia quality assurance and assessment center to develop guidelines and to provide these services to all clinical investigators supported by NCI. The latter group, in conjunction with the Hyperthermia Working Group and the Division of Electronic Products, National Center for Devices and Radiological Health, provide the necessary link between the grant-supported investigators and the extension of hyperthermia research into larger clinical trials to evaluate definitively the role of this promising adjunct to radiotherapy or chemotherapy in the treatment of cancer.

The general radiobiology supported by the Branch encompasses a wide number of areas, the most prominent of which are the study of basic interactions of radiation with matter as well as with biological systems, of basic tumor biology, of the effects of dose fractionation and volume on normal tissues as well as tumors, of the effects and optimization of combinations of radiation and chemotherapeutic agents and of predictive assays of tumor radiocurability. Understanding the more basic effects of radiation alone or in combination with other agents is essential to the improvement of local and regional treatment with radiotherapy.

Although support of research in the area of radiosensitizers and radioprotectors is limited, this area of research is progressing well. A Phase I study coordinated by RTOG has shown that the possible increase in drug dosage with desmethylmisonidazole (NSC #261036) in comparison with misonidazole (NSC #261037) is no more than 10-15% and the limited potential therapeutic gain does not warrant further investigation. However, N-(2-hydroxyethyl)-2-nitro-1H-imidazole-1-atamide the nitroimidazole SR-2508 (NSC #301467) may be considerably more promising. SR-2508 is being evaluated in a Phase I study at this time. Daily doses of 2.7-3.0 gm/m^2, 3 times a week for 3 weeks have been given with no side effects or, at most, minimal nausea and vomiting. This dose, if tolerated on a daily basis 5 days a week for 5-6 weeks, should be adequate to establish whether a hypoxic cell radiosensitizer can influence the outcome of treatment of selected malignancies with radiotherapy.

The Branch is also supporting evaluations of intraoperative radiotherapy (IOR) and of photoradiotherapy (PRT). A working group has been established to develop guidelines for the treatment of intra-abdominal malignancies with IOR. The potential advantage of this technique is that many radiosensitive normal structures can be removed from the radiation field during

the surgical procedure so that a single high radiation dose can be delivered directly to the tumor or tumor bed with minimal risk to the surrounding normal tissues. This approach alone or in combination with a radiosensitizer or with pre- or post-operative external beam radiotherapy promises to give improved control of many localized, yet difficult-to-treat, malignancies. Photoradiotherapy, the use of selected visible light frequencies with hematoporphyrin derivatives, is being investigated by a two-institution working group. The working group is developing guidelines for the use of PRT to include the characteristics and appropriate doses of hematoporphyrin derivatives; the duration, intensity, and method of light exposure; and the temporal relationship of both components of the treatment. Dramatic responses of selected tumors to PRT have been observed and the development of guidelines is essential to the evaluation of this promising modality for the treatment of tumors in numerous sites such as the eye, the head and neck, the lower respiratory tract, the bladder, and other accessible cavities.

In the area of physics, the design study for a heavy ion biomedical research facility is nearing completion and a working group effort to conduct a comparative analysis of treatment planning for tumors with neutrons, protons, pions, helium ions, and heavy ions (as well as with photons) is not only providing the intended information but is upgrading the general capabilities for treatment planning at all particle beam facilities. A major effort to develop methodology for dose calculations for radiolabeled tumor-associated or tumor-specific antibodies has been initiated and will be an important contribution to the exploitation of treatment with labeled antibodies.

VI. Plans for FY84

The RRP will encourage research grant applications as well as develop contract-supported initiatives in a number of the high priority research areas identified by the long-range planning efforts carried out in FY83. In some areas workshops will be held to further refine the goals and objectives of new research initiatives.

Key personnel recruitment activities will include a new Associate Director, a Chief for the Radiotherapy Development Branch, and two professionals for the Low-Level Radiation Effects Branch.

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FY83 Annual Report Summary
Diagnostic Imaging Research Branch

The Diagnostic Imaging Research Branch (DIRB), Radiation Research Program, DCT, NCI, encompasses research on the application of engineering, physical, chemical, biomedical, and physiological sciences to improve diagnostic imaging. This entails research designed for the development of innovative instruments and supportive methodologies in the area of medical imaging as related to patient care.

The research objectives are five-fold. The first objective is to develop new and improved noninvasive imaging devices utilizing both ionizing and non-ionizing radiation technologies to enhance diagnostic imaging for cancer and other diseases and to reduce the exposure of patients to ionizing radiation. The second major objective is to synthesize new radiopharmaceuticals and other compounds in order to advance research in nuclear medicine. The third aim is to produce new nontoxic low cost contrast media. The fourth goal is to develop methodologies for the use of important labeled macromolecules in biological, physiological, and clinical research especially as related to imaging of both normal and malignant tissues. The fifth and most significant aim is the clinical use of the scientific advances for clinical diagnosis and monitoring of therapy.

Overall, the Diagnostic Imaging Program budget for FY82 is $8.7 million, supporting research using grant-in-aid mechanisms. There are 6 program project grants (P01), 57 regular grants (R01) ranging from $45-268 thousands per year, 1 new investigator award (R23), and 1 conference grant (R13). Program projects, ranging from $256-462 thousands in FY82, constitute 22% of the total budget. Nuclear medicine and related research constitute 43% of the budget, instrumentation development 40%, and 17% for other imaging related research.

The instrument development aspect of the program, which constitutes 40% of the total budget, covers almost the whole spectrum of medical imaging with both ionizing and nonionizing radiations. Approximately 87% of the current instrument development research is devoted to the use of ionizing radiations. There are three projects in single photon emission computed tomography (SPECT), two in computed tomography (CT), three in positron emission tomography (PET), five in ultrasound (US), and five in nuclear magnetic resonance (NMR). In addition, there are one or more projects dealing with each of the following topics: slit scanning, 3-D, heavy ion beam imaging, and digital radiography. Other projects deal with algorithms and mathematical models, energy-select diagnostic radiography (EDR), microsphere labeling, and crystal development.

The nuclear medicine research covers 43% of the program (28 projects). Among the topics of interest are: (1) the development of new radiolabeled isotopes using Tc-99m, I-123, Ru-197, Ga-67, Re-168, C-11, and others and (2) research leading to new knowledge in the metabolic pathways in both normal and tumorous tissues.

The remaining 17% of the program budget is devoted to image perception research (6 projects), development of organ-specific contrast agents and new nontoxic contrast media (3 projects), and the bioeffects of diagnostic imaging radiation (3 projects).

The significance of SPECT is that it can give cross-sectional tomographic images with simple gamma-emitting radioisotopes rather than requiring positron emitters as does PET scanning. Because SPECT lacks the coincidence counting based localization of the source of the emitted radiation which is the fundamental aspect of PET scanning, much research is needed to improve both the radiation detection equipment and the software programs for SPECT in order to optimize images. Research in PET scanning, in addition to improvements in imaging equipment, is focusing on the evaluation of labeled metabolic compounds for the study of physiological and biochemical parameters in vivo. Thus rather than stressing pure anatomical information which can often be obtained better with CT, research is going beyond anatomy to study physiological function in states of health and disease.

Nuclear magnetic resonance is a rapidly developing research area that promises to give excellent anatomical images in any body plane as well as information on physiological function. The areas of emphasis in ongoing research include improvements in hardware and software and the development of knowledge of the relationships between the fundamental parameters of NMR imaging (T_1 and T_2 relaxation times) in both normal and diseased tissues.

The new technique of nuclear magnetic resonance (NMR) imaging is the subject of several grants involving the development, application, and evaluation of this new tool for clinical diagnostic uses. Technology for producing high contrast 3D-NMR images has been made available for the generation of images of the breast and other organs. The location of cancerous regions in complete large tissue specimens from radical vulvectomies and from squamous cell carcinomas in lymph nodes have been shown by techniques that can be applied noninvasively and without the use of ionizing radiation.

A large program project is evaluating high magnetic field medical NMR imaging techniques at field intensities of 15 and 30 killogauss. With this latter field the first images of sodium in tissue have been accomplished. Similar images have been achieved with phosphorus imaging as well as with proton density images usually seen with NMR. A spatial resolution of less than 1 mm has been achieved in images of a cat's head, and 1 mm in the human. The potential bioeffects of high magnetic fields are another object of this study.

Studies of phosphorus metabolism by NMR analysis are being carried out to assess the responsiveness of tumors to chemotherapy and radiation therapy. Another NMR spectroscopic study of phosphorus in tissues in vivo is aimed at noninvasively distinguishing between normal and dysplastic or malignant tissues noninvasively by their differences in NMR relaxation times. Phantoms are also being developed specifically for calibration of NMR imaging systems using different gels combined with fats and other additives to simulate the proton spin density and paramagnetic relaxation parameters of biological tissue. Procurement was initiated in FY83 for a contract-supported study of a comparison of NMR with other imaging modalities. The contracts will be awarded early in FY84. The non-ionizing radiation portion of the Diagnostic Imaging Research Program which administers grants and contracts currently involves ultrasound imaging and imaging by nuclear magnetic resonance (NMR). Four investigators are evaluating new types of ultrasound imaging techniques and equipment. Principal emphasis is focused on breast imaging for the detection of cancer and other breast diseases using a computer-assisted ultrasound tomographic approach to produce cross-sectional images of the breast without the use of ionizing radiation.

A principal difficulty with ultrasound tomography is that ultrasonic "rays" do not travel in straight lines but tend to be refracted by the tissues along slightly curved paths, thereby introducing slight distortions and lowering the image resolution. However, corrections for this bending effect have been developed and good progress has been made, particularly with breast images, based on the variation and speed of the ultrasound beam as it passes through different tissues.

Another investigator has been developing artificial breast models, or phantoms, whose materials, shape, and distribution simulate the breast for purposes of calibration of ultrasound breast scanners and for research in ultrasound propagation in the breast. Phantoms developed under this project have already found use in the programs of other investigators and clinicians. A variation of this same model is now under study with different materials to produce a phantom for calibration of NMR images of the breast.

A unique ultrasound imaging system with many transducers spherically focused on a single point is nearing completion. Early images from this system suggest that with further improvement it may be able to characterize tissue noninvasively, including breast tissues. Basic research is also under way to study the bioeffects of in vivo cavitation involved in tissues at therapeutic (high power) levels of ultrasonic energy. Threshholds of ultrasonic intensity required to produce in vivo cavitation are being studied as well as their effects on platelet function, blood coagulation, and organ histology. Although there are presently no known hazards from the clinical use of ultrasound at diagnostic power levels, a study is presently under consideration to widen the data base regarding potential effects, if any, in tissues.

One of the most promising techniques to be developed in recent years in radionuclide tracer methodology is the use of monoclonal antibody (MCA) in diagnostic imaging, immunotherapy, and immunochemotherapy. Advances in the hybrid cell culture techniques make it possible to produce highly specific MCA. In the past few years, antibodies to melanoma neuroblastoma and cancer of the colon, lung, and breast have been described. The efficacy of monoclonal antibody fragments as carriers of radioisotopes for diagnostic imaging or for radiotherapy is a highly promising field of research. Our program is very much interested in encouraging research in this important area. Presently, we support one project and have a pending program project application in MCA tracer investigations.

The ultimate aim of the Diagnostic Imaging Research Program is to develop instruments and techniques characterized by enhanced imaging resolution, reduced ionizing radiation and time of exposure, and lowered cost to patients to better enable the physician to safely diagnose and treat cancer and other diseases.

FY83 Annual Report Summary
Low-Level Radiation Effects Branch

The Low-Level Radiation Effects Branch (LLREB) is concerned with research that will provide new and relevant information on the molecular and cellular processes leading to mutagenesis, cell transformation, and carcinogenesis by ionizing radiation, in particular at low doses or low dose rates. Both cellular and whole animal studies are used, but fundamental biophysical and radiochemical investigations are also supported as well as special epidemiological studies. The LLRE program was established in direct response to Public Law 95-622.

In the area of human radiobiology, an epidemiological study was initiated in FY 82 for the "Assessment of Leukemia and thyroid Disease in Relation to Fallout in Utah". This contract includes 1) a study of milk consumption patterns in relation to milk sources and fallout exposures, 2) a case-control study of thyroid cancer in Utah, 3) a cohort study of malignant and benign thyroid disease, 4) a case-control study of leukemia in Utah, and 5) a cohort mortality study of leukemia in Utah. The Department of Energy and the Department of Defense participate in the funding of this contract. Two follow-up studies are designed to estimate the risk of human thyroid cancer from radiation exposures. One is a study of patients who had iodine-131 and other diagnostic procedures during childhood and adolescence, while the other is a continuing study of patients with a history of external irradiation to the head and neck during childhood.

The LLRE Branch provides grant support to the National Council on Radiation Protection and Measurements (NCRP), the International Commission on Radiation Units and Measurements (ICRU) and the International Commission on Radiological Protection (ICRP). These distinguished advisory bodies have published many important reports and recommendations, and the NCRP has drafted a timely report on thyroidal carcinogenesis following exposure to ionizing radiation.

Several studies supported by the LLRE Branch concern the induction of malignancies in mice by ionizing radiation. In one of these, the experimental protocol is to irradiate different strains of mice with single doses, repeated doses and with a wide range of dose rates to determine: 1) the incidence of leukemia at low average dose rates; 2) the presence of preleukemic cells in the mice as a function of total dose and dose rate; 3) the number of preleukemic cells initiated by radiation; 4) the relative degree of "repair" following single, repeated, and continuous exposure; and 5) if there remains a fraction of the effects of low-LET radiation that is nonreparable, or comparable to the "single hit" damage and effects seen with high LET-radiation. In another study, radiation-induced myelogenous leukemia from fission neutrons is being observed in mice to establish a reliable dose-response function as well as to relate induction of malignancy to the production of chromosome aberrations. Two additional studies in rodents involve induction of cancer by ionizing radiation in combination with a second agent.

Two projects investigate the variation in sensitivity to ionizing radiation as a function of stage of development. One of these is an evaluation of the types and frequencies of neoplasms occurring in dogs that have previously been irradiated at various times during development. Groups of dogs have been exposed to whole-body irradiation at three prenatal and three postnatal ages, specifically at 8, 28 or 55 days postcoitus, and at 2, 70 or 365 days postpartum at doses of either 16 or 83 rad. As of the end of December, 1982, there were about 630 dogs remaining alive, all of them at least 10 years old. It is estimated that 50-75% of these will die of some malignancy in future years. Preliminary projection analysis indicates that the greatest sensitivity to tumor induction occurs just prior to and just after birth. A study in rodents addresses primarily teratogenesis and other embryo toxic effects, but also will include an evaluation of leukemia induction in prenatally exposed mice. Unique differences between the effects of X-irradiation and various energies of neutron radiation have been demonstrated.

The effects of ionizing and ultraviolet radiation on cells are being studied in a wide variety of cell types from humans, Chinese hamsters, rats, mice, plants and bacteria. Each cell type offers certain advantages for studying unresolved aspects of cell killing, malignant transformation and mutation. A long-term goal of this research program is to gain knowledge about mechanisms which determine the radiosensitivity of mammalian cells, including those involved in the response of stationary or very slowly proliferating cells to X-irradiation. One study is using three different types of cells: 10T 1/2 mouse embryo fibroblasts, human diploid fibroblasts (HDF) and human tumor cells. Five cellular endpoints are being studied in parallel: survival, transformation, mutagenesis and the induction of chromosome aberrations and sister chromatid exchanges (SCE). The relationships between these cellular effects are being investigated, and attempts will be made to correlate them with molecular DNA repair processes. Techniques for measuring DNA repair include host cell reactivation of irradiated herpes virus, alkaline elution assay for DNA strand breaks and crosslinking, and measurement of endonuclease sensitive sites. In another study, emphasis is on defining the effects of ultraviolet and X-radiations on arrested nonmitotic populations of various HDF strains including the DNA repair deficient xeroderma pigmentosum (XP) and ataxia telangiectasia strains and determining the rate and extent of DNA repair in these arrested populations. Another investigation is on the interaction of UV-C light and X-rays in the induction of neoplastic transformation of 10T 1/2 cells. Exposure of cells to a UV-C dose leaving 80% survival, followed immediately by graded doses of X-rays, results in synergistic interaction in induction of neoplastic transformation. However, when the order of exposures is reversed, only the effect of the X-ray dose is expressed.

A team of investigators has developed a reproducible, quantitative assay for carcinogen-induced mutation or neoplastic transformation of diploid human fibroblasts. They plan to use these assay systems to determine the response of such cells to the transforming action and mutagenic action of low doses of ionizing radiation. They will further determine if repair of sublethal or potentially lethal damage influences these effects of the radiation, or if cells from cancer prone individuals respond differently.

A significant observation has been made that X-rays induce a stable hypermutable state in cultured hamster fibroblasts. The further establishment of this phenomenon and its more detailed characterization in a variety of cell types including human diploid cells, is being pursued. If the hypermutable state proves to be an intermediate in the transformation progression, it should prove to be a very useful marker in elucidating the mechanisms involved in this process as well as in carcinogenesis.

One research group is investigating the UV light-induced transformation of cultured human cells, on DNA damage and repair in cultured cells and in skin, and the relation of these areas to solar oncogenesis in man. The investigators have developed and characterized a system for transformation of primary human embryonic skin and muscle fibroblasts (HESMs) using anchorage-independent growth in agar as the indicator for transformation. They have shown that transformation frequency is related to dose, wavelength, number and spacing of irradiations, cell passage number and duration of growth period prior to plating in agar. In subsequent monolayer culture, the transformed cells show characteristic loss of contact and density inhibition.

The aim of one study is to gain further information on the number, nature and radiation response of cells involved in mammary carcinogenesis. A survival assay for transplanted mammary cells has been developed in which mammary cells from a donor rat are transplanted to fat pads in recipient rats and the number of alveolar units which develop is observed. Since the situation is similar to a tumor transplant experiment, there are a wide variety of experimental protocols which can be attempted. In addition to short term survival studies, long term carcinogenesis studies using both in situ and cell transplantation methods are being initiated.

In one low-level radiation study, the dose-response curve has been established for stamen hair somatic mutation in the Tradescantia plant following exposure to ^{137}Cs gamma rays and has confirmed the hypothesis of linearity below about 7.5 rad. The results of chronic and fractionated gamma exposures support the hypothesis that with low-level radiation, the mechanism of action is exclusively that of the high LET component of the radiation.

NCI is supporting one of the very few projects in the world which attempts to look at rapid kinetics of chemically induced changes in cellular radiosensitization. This project is concerned with obtaining some understanding of the early physicochemical events involved in radiation effects in cells. The approach employed, based on experiments using a field emission accelerator as a radiation source, is to measure the kinetics and temperature dependence associated with changes of radiation sensitivity in untreated cells. The radiation sensitivity is then manipulated by the addition of various agents such as sensitizers, protectors, or D_2O. Through the measurement of the kinetics and temperature dependence of the treated cells, it is then possible, to determine not only the effect on the early events, but to gain knowledge of the action of the agents. Spores, bacteria and mammalian cells are being used. Experiments have also been planned to examine the influence of LET on the temperature and water dependence of radiosensitivity through irradiation of bacteria or spores with particles from a cyclotron.

Another part of the program focuses on the identification of initial radiation damage to DNA, its repair and factors that may influence this damage and repair at the chromosomal level. The primary emphasis is on ionizing radiations but some projects involving ultraviolet radiation are also included. A number of investigators are studying the various aspects of DNA repair. One of these involves the characterization of the enzymes and reactions involved in excision repair of DNA in human cells exposed to either radiation or alkylating agents. These efforts focus on the critical first step in excision, namely recognition of specific lesions by repair endonucleases or N-glycosylases. Attempts are being made to further purify the already 1000-fold purified 3-methyladenine-DNA glycosylase from human placenta.

The importance of the chromosomal structure and function for the sensitivity of specific regions of the DNA is addressed in two projects. In one of these, the objective is to compare transcriptionally active and inactive chromatin with respect to initial yields of ionizing radiation-induced DNA damage and to repair capacity. Specific model systems will be ribosomal chromatin, which contains the DNA coding for ribosomal RNA, chromatin containing genes active in messenger RNA synthesis, and chromatin containing inert DNA. A system has been developed to analyze single-strand breaks in the specific DNA region: DNA is sized by agarose gel electrophoresis or single- and double-stranded DNAs are separated on hydroxyapatite after partial unwinding in alkali, then each fraction is probed by hybridization to DNAs or RNAs complementary to the region of interest.

The involvement of DNA as a target in carcinogenesis is addressed in a study to test the hypothesis that enhanced DMBA tumorogenesis in hamster cheek pouch by X-radiation is due, wholly or in part, to the radiation induction of alterations in the binding of DMBA to the DNA of the target epithelial cells and to test the applicability of *in vitro* results to the *in vivo* situation by performing parallel studies on the cheek pouch grown as an organ culture and *in situ* in the animal. These studies may potentially yield information on the mechanism of interaction between radiation and chemical carcinogens in animal tumor production.

A final three projects involve the effects of UV radiation and the repair of UV-induced damage in DNA. Both the short-term and long-term effects of UV on DNA synthesis are being studied in CHO cells and human cells. It has been found that the UV-sensitive UV5-CHO cells exhibit a much slower recovery of DNA synthetic rates and DNA chain growth than do the wild-type AA8-CHO cells.

FY83 Annual Report Summary
Radiotherapy Development Branch

The Radiotherapy Development Branch (RDB) is by far the largest component of the RRP. It administers a large program of basic, developmental, and clinical research related to cancer treatment modalities utilizing ionizing or nonionizing radiations and the investigation of means of modifying the biological effects of these radiations. This body of research covers a range of scientific disciplines including radiation biology, radiation chemistry, radiation physics, and radiation oncology. Research efforts range from the investigation of basic interaction mechanisms between radiation and biological systems to controlled clinical trials for a multitude of disease sites treated with single or multi-modality treatment schemas. Research in radiation modifiers includes both radioprotective agents which reduce normal tissue morbidity, radiosensitizers which enhance the effects of radiation on tumors but not in normal tissues, and hyperthermia which also enhances the effects of radiation on tumors compared to normal tissues.

Many areas of basic research which have been supported by RDB have resulted in new treatment modalities that have now been implemented as new cancer therapies or are currently being tested in clinical trials. Among these are heavy particle therapy using neutrons, protons, helium ions, and heavy ions such as carbon, neon, and silicon; hyperthermia used alone or in conjunction with radiotherapy; chemical modifiers used as radioprotectors and radiosensitizers; and photoradiotherapy.

The largest areas of research in terms of funding are heavy particle radiotherapy, hyperthermia, and general radiobiology. Support of research in the development of radiosensitizers and radioprotectors, intraoperative radiotherapy, photoradiotherapy, and physics have also been important activities of the Branch in FY83.

The heavy particle radiotherapy research activities are multifaceted and include strong radiobiology and physics, as well as clinical research, components. In the area of physics, the design study for a heavy ion biomedical research facility is nearing completion and a working group effort to conduct a comparative analysis of treatment planning for tumors using neutrons, protons, pions, helium ions, and heavy ions (as well as with photons) is not only providing the intended information but is upgrading the general capabilities for treatment planning at the heavy particle facilities. Clinically dedicated neutron therapy facilities are being completed at the Fox Chase Cancer Center, M. D. Anderson Hospital, and the University of Washington. Progress at the University of California, Los Angeles, has been delayed slightly by the bankruptcy of the manufacturer of the cyclotron. Encouraging preliminary results are being reported from the existing neutron therapy facilities in studies coordinated by RTOG on malignant gliomas, neck nodes, prostatic carcinoma, and bladder cancer. Very promising results are being observed at the Lawrence Berkeley Laboratory and the Harvard cyclotron in the treatment of posterior uveal melanomas with helium ions and protons, respectively. Treatment of other selected tumors at these facilities have convincingly demonstrated the dose localization advantages of protons and helium ions. The follow-up evaluation of patients treated with pions at Los Alamos continues even though treatment at the facility stopped in May of 1982.

Interest in the potential use of hyperthermia as a potent modifier of the response of tumors to radiation and chemotherapy has led to an increasing number of grant-supported research projects in this area. Investigations now supported include the study of basic effects of heat alone or in combination with radiation on DNA crosslinks, membranes, cells, tumors and their blood vessels, normal tissues and the immunological responses; of the effects of blood flow, the cellular environment, and the development of tolerance to heat, and of the duration and temporaral relationship of the heating to the radiation; and of the development of methodology for heating (extra corporeal, interstitial, external) and for thermometry. The RDB is supporting by contract a working group of five leading institutions in this country to evaluate equipment available for heating cancers and for measuring temperatures therein and to develop guidelines for the use of this equipment by other investigators. A contract has been awarded to establish a hyperthermia quality assurance and assessment center to develop guidelines and to provide these services to all clinical investigators supported by NCI. The latter group, in conjunction with the Hyperthermia Working Group and the Division of Electronic Products, National Center for Devices and Radiological Health, provide the necessary link between the grant-supported investigators and the extension of hyperthermia research into larger clinical trials to evaluate definitively the role of this promising adjunct to radiotherapy or chemotherapy in the treatment of cancer.

A contract has been funded to study the dose calculations for cancer therapy using radioactively labeled antibodies directed to tumor-associated and/or tumor-specific antigens. One of the most severe limitations in the delivery of tumorcidal radiation doses is the risk of injury to normal tissue surrounding the target volume. The ideal radiation therapy system would be radioisotopes attached to tumor specific antibodies which would concentrate the radiation dose in the tumor cells whether in the primary tumor or in distant metastases. The advances in the development of monoclonal antibodies as well as technology in radioactively labeling tumor-associated/specific antibodies have set the stage for the advance of this area of investigation to the clinical testing phase.

The general radiobiology supported by the Branch encompasses a wide number of areas the most prominent of which are the study of basic interactions of radiation with matter as well as with biological systems, of basic tumor biology, of the effects of dose fractionation and volume on normal tissues as well as tumors, of the effects and optimization of combinations of radiation and chemotherapeutic agents, and of predictive assays of tumor radiocurability. Understanding the more basic effects of radiation alone or in combination with other agents is essential to the improvement of local and regional treatment with radiotherapy.

Although support of research in the area of radiosensitizers and radioprotectors is limited, this area of research is progressing well. A Phase I study coordinated by RTOG has shown that the possible increase in drug dosage with desmethylmisonidazole (NSC #261036) in comparison with misonidazole (NSC #261037) is no more than 10-15% and, therefore, the limited potential therapeutic gain does not warrant further investigation. However, another nitroimidazole - SR-2508 (NSC #301467) - may be more promising. SR-2508 is being evaluated in a Phase I study at this time. Daily doses of 2.7-3.0 gm/m^2 3 times a week for 3 weeks have been given with no side effects or, at most, minimal nausea and vomiting. This dose, if tolerated on a daily basis 5 days a week for 5-6 weeks, should be adequate to establish whether a hypoxic cell radiosensitizer can influence the outcome of treatment of selected malignancies with radiotherapy. The RDB is also supporting

contract efforts in the synthesis of new radiosensitizers. The current contractors have synthesized most of the 400 plus components they have evaluated to date. In the process, a better understanding of the relationship between molecular structure, physio-chemical parameters, and radiobiological activity was realized. Using this information, SR-2508, the optimal radiosensitizer of the nitroimidazole class, was developed. Other important leads have been uncovered which will ultimately result in the rational design and development of new, noval, non-nitro classes of radiosensitizers. Until now, only one class of chemical compounds have been evaluated as sensitizers (nitroimidazoles) and as radioprotectors (aminoalkylthiols). It is important to screen other classes of chemical compounds to determine whether any of them show potential as radiosensitizers or protectors. A current contract effort will provide the capability of detecting new leads which could eventually lead to the development of more effective radiosensitizers and radioprotectors. Approximately 250 compounds representing various classes and structures have been screened to date in both the sensitizer and the protector screens. Some of these compounds appear as effective as the standards to which they are compared but they represent different classes in which more effective analogues could be developed. It is too early to evaluate the effect of the current screening contracts, but the potential they represent make them worthy of continuation.

The RDB is also supporting an evaluation of intraoperative radiotherapy (IOR) and of photoradiotherapy (PRT). A working group has been established to develop guidelines for the treatment of intra-abdominal malignancies with IOR. The potential advantage of this technique is that many radiosensitive normal structures can be removed from the radiation field during the surgical procedure so that a single high radiation dose can be delivered directly to the tumor or tumor bed with minimal risk to the surrounding normal tissues. This approach alone or in combination with a radiosensitizer or with pre- or post-operative external beam radiotherapy promises to give improved control of many localized, yet difficult-to-treat, malignancies. Photoradiotherapy, the use of selected visible light frequencies to activate hematoporphyrin derivatives, is being investigated by a two-institution working group. The working group is developing guidelines for the use of PRT to include the characteristics and appropriate doses of hematoporphyrin derivatives; the duration, intensity, and method of light exposure; and the temporal relationship of both components of the treatment. Dramatic responses of selected tumors to PRT have been observed and the development of guidelines is essential to the evaluation of this promising modality for the treatment of tumors in numerous sites such as the eye, the head and neck, the lower respiratory tract, the bladder, and other accessible cavities.

The RDB supports research through the mechanism of grants and contracts. In FY84, multi-institutional programs such as neutron therapy, hyperthermia therapy, and intraoperative therapy will be converted to cooperative agreements. The RDB currently administers a contract-supported research portfolio of about 25 contracts at a funding level of approximately $5 million and a grant-supported research program consisting of 165 grants at a funding level of approximately $43 million.

There are four types of grants: R01's, P01's, R13's, and R23's. The R01 type grants are relatively small in dollar amounts (average of $135 thousand per year each) and are usually single projects directed toward specific research problems. The R01 grants in the area of radiobiology are designed to support the study of a broad spectrum of the effects of radiation on molecules, especially DNA, or

normal and tumor cells (in vitro and in vivo), or on animal models. There are currently 132 R01 grants totaling about $18 million per year.

The P01 type grants are relatively large in dollar amounts (average of $1.09 million per year each) and are usually composed of multiple projects directed toward a central idea or unifying theme. The typical P01 grant is directed toward the evaluation of heavy charged particle therapy, or multi-modality therapy (heat, radiation and drugs), and usually includes limited clinical trials in various disease sites. These large programs generally have projects covering several disciplines; e.g., biology, physics, therapy, and statistics. There are currently 22 P01 grants totaling about $24 million per year.

There are ten R23 type grants in the RDB for a total of about $500,000. These grants are new investigator research awards. Finally, there is one conference grant for $93,000.

The RDB plans to continue to encourage and support innovative basic research directed toward eludication of the basic mechanisms of interaction between radiation and biological systems as well as the modification of these interactions by heat or chemical modifiers. When basic studies provide the necessary information, RDB will actively pursue the testing in appropriate models of potential new or adjunct therapies, and finally, will promote the advance of those agents which show positive effects into the clinical testing phase. This "total systems" approach, from the embryonic laboratory experiment to the curative treatment of previously intractable cancer, has proven to be a successful approach in cancer management.

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