Division of

CANCER TREATMENT

1985 Annual Report Volume I
October 1, 1984-September 30, 1985

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

National
Institutes of
Health

National
Cancer
Institute

Bethesda,
Maryland 20892

NATIONAL CANCER INSTITUTE

ANNUAL REPORT

October 1, 1984 through September 30, 1985

CONTENTS

ANNUAL REPORT

DIVISION OF CANCER TREATMENT

October 1, 1984 through September 30, 1985

The Division of Cancer Treatment (DCT) is the organizational component of the National Cancer Institute (NCI) responsible for the development and evaluation of new therapies for the control and cure of cancer. The program is carried out in intramural laboratories and clinics as well as through grant-contract and cooperative agreement-supported projects throughout the nation and the world. Major emphasis in the treatment research program is on optimal and integrated use of all modalities of therapy, including chemotherapy, surgery, radiotherapy, hyperthermia, biological response modifiers, and immunotherapy, used both individually and in combination.

The Division has five major programs: the Developmental Therapeutics Program (DTP), the Cancer Therapy Evaluation Program (CTEP), the Radiation Research Program (RRP), the Clinical Oncology Program (COP), and the Biological Response Modifiers Program (BRMP). Each of these programs has a separate and distinct responsibility, encompassing research on the development and identification of new agents or methods for the treatment of cancer; their evaluation in preclinical tumor systems; the testing and evaluation of the safety of new agents or methods in preparation of investigational new drug applications (INDs) for submission to the Food and Drug Administration for approval to initiate clinical trials; the conduct of clinical trials at the earliest level (Phase I); the conduct of disease-specific clinical trials (Phase II); the conduct of clinical trials comparing new and standard treatments (Phase III); and the evaluation of combined modalities of therapy (Phase IV).

For scientific and administrative direction, the Division relies heavily on the advice of its Board of Scientific Counselors, whose membership and affiliations are shown in Table I. The Board represents outstanding extramural scientific talents in the fields of surgical oncology, immunology, and radiotherapy. These represent the areas of clinical science particularly relevant to the research mission of the Division.

The Division Director relies on the Board of Scientific Counselors not only for scientific and administrative advice but also for fiscal direction. For example, all new concepts for contracts and Requests for Application (RFAs) must meet with Board approval. In addition, Board members serve as chairpersons and members of regular site visits to intramural labs. The Board's counsel is invaluable in assuring that the best intramural labs receive appropriate support while areas of lower scientific priority can be de-emphasized.

Personnel and Organization

The DCT is operationally divided in the five major components of treatment program priorities. Each program is headed by an Associate Director who is responsible for the overall direction of the science within that program. A current organizational chart, shown in the following pages, reflects changes that have occurred during the past year. Restructuring within the DTP consolidated the Laboratory of

TABLE I. DCT BOARD OF SCIENTIFIC COUNSELORS

Name	Affiliation	Term of Appointment
Dr. Samuel A. Wells, Jr.	Washington University School of Medicine	1982-1986
Dr. Dani P. Bolognesi	Duke University Medical Center	1982-1986
Dr. David G. Bragg	University of Utah Medical Center	1982-1985
Dr. Paul Calabresi	Roger Williams General Hospital, Brown University	1982-1986
Dr. Mortimer M. Elkind	Colorado State University	1982-1986
Dr. Karen K. Fu	University of California, San Francisco	1983-1985
Dr. James Goldie	A. Maxwell Evans Cancer Control Agency of British Columbia	1982-1985
Dr. I. David Goldman	Medical College of Virginia	1982-1986
Dr. Leon Goodman	University of Rhode Island	1982-1986
Dr. Robert L. Goodman	University of Pennsylvania	1984-1987
Dr. Susan B. Horwitz	Albert Einstein College of Medicine	1982-1986
Dr. John H. Kersey	University of Minnesota Hospitals	1984-1985
Dr. Rodrigue Mortel	Milton S. Hershey Medical Center, Pennsylvania State University	1983-1985
Dr. Carol S. Portlock	Yale University School of Medicine	1982-1985
Dr. Efraim Racker	Cornell University	1982-1986
Dr. Alan S. Rosenthal	Merck Sharp and Dohme Research Labs.	1984-1986

Experimental Therapeutics and Metabolism, Laboratory of Chemical Pharmacology, and Laboratory of Medicinal Chemistry into the Laboratory of Biological Chemistry and the Laboratory of Pharmacology and Experimental Therapeutics. In addition, there were several changes in personnel:

A. Office of the Director (OD)

- Dr. Arnold Welch, Acting Deputy Director, left the OD to assume responsibilities coordinating the newly established National Cooperative Drug Discovery Groups within the Developmental Therapeutics Program.

- Dr. Gregory Curt assumed responsibilities as Deputy Director.

- Dr. Marcia Browne transferred from the COP to become Special Assistant to the Director for Clinical Affairs.

- Dr. Eddie Reed transferred from the DTP to become Special Assistant to the Director for Science.

- Dorothy Tisevich assumed responsibilities as Deputy Administrative Officer, OD, DCT.

B. Biological Response Modifiers Program (BRMP)

- Dr. Dan L. Longo left the COP to assume new responsibilities as the Associate Director, BRMP.

- Dr. Ronald Herberman left as Acting Associate Director, BRMP, and Chief of the Biological Therapeutics Branch to become head of the Cancer Center at the University of Pittsburgh.

- Dr. Carl Pinsky was recruited as Chief of the Biological Resources Branch.

- Dr. Freddie Hoffman left the Clinical Investigations Branch, CTEP, to become Head of the Clinical Trials Section, Biological Resources Branch.

- Gordon Cecil became the new Administrative Officer for BRMP.

C. Cancer Therapy Evaluation Program (CTEP)

- Dr. Brigid Leventhal spent a sabbatical year from Johns Hopkins University as Special Assistant to the Associate Director, CTEP.

- Dr. Edward T. Jacobs retired from government service. Drs. Silvia Marsoni, Mercedes Lassus, Elihu Estey, and Elizabeth Read left the CTEP for positions elsewhere, to be replaced by Drs. Bruce Cheson, Hoo Chun, Gisele Sarosy, and F. Andrew Dorr; Drs. Michael Stewart, and J. Michael Hamilton also joined the staff.

- Dr. Michael Hawkins was recruited from the University of Wisconsin as Head, Biologics Section, Investigational Drug Branch, and Dr. Marianne Roper joined the staff of the Biologics Section.

- Dr. Lawrence Rubinstein joined the staff of the Biometric Research Branch.

- Drs. Allison Martin and Stacey Nerenstone joined the CTEP staff after finishing clinical fellowship training in the Medicine Branch, COP.

D. Clinical Oncology Program (COP)

- Dr. Neil Rosen was appointed a Senior Investigator in the Medicine Branch.

- Kathy Russell became the new Administrative Officer for COP.

E. RADIATION RESEARCH PROGRAM (RRP)

- Robert Morton was recruited from the Food and Drug Administration to join the Radiotherapy Department.

- Barbara Vermillion became the new Administrative Officer for RRP.

International Treatment Research

International treatment research activities of the Division are coordinated through the Office of the Director. Dr. Michael Friedman, Chief of the Clinical Investigations Branch, CTEP, has continued to coordinate the Japanese-American Bilateral Agreement. Two meetings were held during the past year, the first in Tokyo (Workshop on Biochemical Modulation of Chemotherapy and Immunotherapy) and the second in Hawaii (New Drug and Biological Response Modifier Development). The Italian and French bilateral agreements were administered by Dr. Gregory Curt. The French met with American colleagues in Bethesda this year to exchange information on current concepts in bone marrow transplantation. The proceedings of this meeting will be published in CANCER TREATMENT SYMPOSIUM. The Italian-American Bilateral Agreement met in Venice to exchange information on drug resistance, differentiating agents, and the quality of life following cancer therapy. Administration of the agreements with the People's Republic of China, Egypt, and Poland were assumed by Dr. Marcia Browne.

Most importantly, the agreements continued to sponsor individual exchanges of scientists on a laboratory-to-laboratory basis to enhance international exchange of reagents, techniques, and ideas.

Cooperative relationships with European cancer researchers and drug industries have been further strengthened by the Division's relationship with the EORTC (European Organization for Research on Treatment of Cancer). This interaction continues to have both preclinical and clinical components. In order to identify new agents useful in the treatment of cancer, drugs collected in Western Europe are screened at the Institut Jules Bordet in accordance with NCI protocols. In addition, important clinical programs are sponsored by this agreement. The EORTC has become a major testing ground for Phase I and II agents. Continued exchange will allow the expedited access of the best new anticancer drugs on both sides of the Atlantic.

The EORTC and NCI are also sponsoring a new training program that will allow postdoctoral fellows from Western Europe and Israel as well as American colleagues to spend significant research time in the laboratories of like-minded scientists. Candidates will spend three to five years in sponsoring exchange laboratories before returning to their home institutions.

Scientific Accomplishments

I. Training Programs

In addition to the EORTC training program, the Office of the Director implemented a traineeship program in oncology nursing. This initiative was implemented by Dr. Marcia Browne in close collaboration with the Nursing Department of the Clinical Center, NIH. The purpose of this new nine-month training program is to prepare recent baccalaurate nursing graduates to become skilled oncology nurse

practitioners through a unique program emphasizing both practical and theoretical aspects of cancer nursing. This represents the first program of its type in the United States to recognize the central role of the nurse as a team member in the treatment of cancer patients. It is expected that this program will expand and diversify to include a research fellowship for oncology nurses interested in the design and implementation of clinical trials. Training will include trial design, biostatistics, biochemistry, pharmacology, and immunology. It is hoped that this program will serve as a model for similar initiatives throughout NIH and the nation.

In addition, the Office of the Director coordinated a new fellowship program in biotechnology. This program, established under the training authority of the National Cancer Act, will allow American citizens to pursue exciting new opportunities in molecular biology, genetic engineering, immunology, and molecular pharmacology.

II. Surgical Oncology

Recognizing the importance of the surgeon in the treatment of cancer patients and realizing a shortage in academically trained research-oriented surgical oncologists, the Office of the Director has worked with Dr. Fred Avis, Section Head for Surgical Oncology, and the Division of Cancer Prevention and Control to establish a new program for the development of a cadre of cancer surgeons with broad expertise in cancer biology and treatment.

The first of these initiatives was the establishment of the Clinical Investigator Award (K08), a premiere support mechanism for the provision of long-term basic and clinical research training for M.D.s. Surgical oncology is targeted for special priority for K08 awards.

Most recently, Drs. Fred Avis and Barney Lepovetsky of the DCT Cancer Training Branch have collaborated in the establishment of a Surgical Oncology Training Program that provides maximal flexibility within the existing National Service Research Awards mechanism to allow surgeons meaningful subspecialty training in surgical oncology research.

In order to improve surgical representation on study section rosters, the OD distributed detailed information on NCI initiatives in surgical oncology, the NCI grants process, and the importance of review to the entire membership of the Society of Surgical Oncology. Finally, in order to follow the progress of these initiatives, surgical oncology has been designated a Cancer Activity, which will allow accurate tracking of the budget in this area of cancer treatment.

III. Unproven Methods of Cancer Treatment

Dr. Curt was appointed to the Unproven Methods Committee of the American Cancer Society. In that capacity, he worked with extramural investigators, the Centers for Disease Control, and the Pan American Health Organization to investigate the practice of Dr. Lawrence Burton, who had treated 3,000 cancer patients with immunoaugmentative therapy, an unproven cancer treatment, at his clinic in Freeport, Grand Bahamas. In addition to determining that this treatment was without biological activity, it was discovered that treatment materials were contaminated with hepatitis B and HTLV-III antibodies. This information led to the closing of the clinic in July 1985.

IV. HTLV-III

It has been conclusively demonstrated that HTLV-III is the etiologic agent of acquired immunodeficiency syndrome (AIDS). The Office of the Director was instrumental in competing and commercializing the ELISA assay for the screening of HTLV-III in blood products. The massive screening program that resulted protected the nation's blood resources from iatrogenic spread of the disease.

V. Special Initiatives

Working together with the Comprehensive Minority Biomedical Program of the Division of Extramural Activities, the Office of the Director established a minority supplement program for the Clinical Cooperative Groups. These awards will improve access of minority patients to state-of-the-art clinical trials. This initiative is considered particularly important as survival statistics for minority patients with cancer have continued to lag behind the steadily improving survival for American whites.

The Office of the Director, DCT, worked with the NIAID and the NHLBI to formulate a technology assessment conference for the Office of Medical Applications of Research that recommended deferring establishment of a national registry for unrelated bone marrow transplantation.

Detailed preclinical and clinical research accomplishments will be described under each of the Division's program annual reports.

Publications

Chabner, B.A., Curt, G.A. and Hubbard, S.M.: Surgical oncology research development: the perspective of the National Cancer Institute. Cancer Treat. Rep. 68: 825-829, 1984.

Chabner, B.A., Wittes, R., Hoth, D. and Hubbard, S.: Investigational trials of anticancer drugs: establishing safeguards for experimentation. Public Health Rep. 99: 355-360, 1984.

Chabner, B.A., Fine, R.L., Allegra, C.J., Yeh, G.C. and Curt, G.A.: Cancer chemotherapy: progress and expectations, 1984. Cancer 54: 2599-2608, 1984.

Chabner, B.A.: The evolution of cancer chemotherapy. Hospital Practice 20: 115-127, 1985.

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 FY85

	ANNUAL LEVEL	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	34,357,909	63.42
STAGE I	8,011,385	14.79
1XX ACQUISITION OF MATERIALS		
STAGE IIA	9,722,009	17.95
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	8,537,885	15.76
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	702,696	1.30
2B2 DETAILED EVALUATION OF NEW AGENTS	3,561,410	6.57
2B3 PROCURE. OF AGENTSS FOR PRECLIN. STUDIES	4,273,779	7.89
STAGE III	8,086,630	14.92
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	2,684,609	4.95
3X2 PROD. & FORM. FOR CLINICAL TRIALS	5,402,021	9.97
PBR PRECLINICAL BASIC RESEARCH	4,226,524	7.80
PBS CELLULAR/SUBCELLULAR STUDIES	1,427,422	2.63
PTS TREATMENT STUDIES	2,799,102	5.17
CTR CLINICAL TRIALS RESEARCH	9,792,311	18.09
CT1 PHASE I CLINICAL TRIALS	3,035,358	5.61
CT2 PHASE II CLINICAL TRIALS	1,433,494	2.65
CT3 PHASE III CLINICAL TRIALS	465,165	.86
CT5 OTHER CLINICAL TRIALS RESEARCH	4,858,294	8.97
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	2,464,842	4.55
STATISTICS, DATA PROCESSING, AND OTHER CLINICAL TRIALS RESEARCH	213,726	.39
MGT PROGRAM MANAGEMENT	3,117,157	5.75
TOTAL	54,172,469	100.00

Table II

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	Alabama, University of	N CM2757100	129,181
D.T.P.	Alabama, University of	N CM3763105	30,648
D.T.P.	Alabama, University of	N CM3763106	17,984
D.T.P.	Alabama, Univeristy of	N CM3763107	12,126
D.T.P.	Alabama, University of	N CM3763108	12,808
D.T.P.	Biotech Research Laboratories, Inc.	N CM3755800	40,434
I.S.D.T.	Biotech Research Laboratories, Inc.	N CM3760900	302,933
D.T.P.	Bristol Laboratories	N CM3755600	364,560
A.P.	Charles River Breeding Labs.	N CM3762600	593,050
A.P.	Charles River Breeding Labs.	N CM8721200	5,600
A.P.	Charles River Breeding Labs.	N CM9722900	13,362
B.R.M.P.	Chemical Coupling of MoAb	RFP57718000	400,000
D.T.P.	Dyanamac Corporation	N CM3756300	111,454
D.T.P.	Fein Marquart	N CM4768300	15,175
D.T.P.	Flow Laboratories, Inc.	N CM2750500	280,071
D.T.P.	Georgia Institute of Technology	N CM2751700	149,236
A.P.	Harlan Sprague Dawley, Inc.	N CM3762300	104,066
A.P.	Harlan Sprague Dawley, Inc.	N CM2391100	750,000
B.R.M.P.	Harlan Sprague Dawley, Inc.	N CM2391100	99,825
D.T.P.	Illinois, University of	N CM3751300	113,380
D.T.P.	Institute of Microbial Chemistry	N CM4759300	130,000
A.P.	Leo Goodwin Inst. for Cancer Research	N CM3762500	69,000
D.T.P.	Maxima Corporation	N CM4762800	76,839
B.R.M.P.	Meloy Laboratories, Inc.	N CM4765000	203,612
A.P.	Microbiological Associates	N CM9728700	70,144
A.P.	Missouri, University of	N CM2753400	16,028
A.P.	Missouri, University of	N CM8715700	34,632
A.P.	Murphy Breeding Labs., Inc.	N CM3022900	1,835
A.P.	Northwestern University	N CM3753800	25,676
A.P.	Papanicolaou Cancer Research Inst.	N CM8723000	14,895
D.T.P.	Polysciences, Inc.	N CM3755700	217,923
B.R.M.P.	Production of Hybridomas	RFP57720	175,000
A.P.	Program Resources, Inc.	N C02391000	810,280
A.P.	Simonsen Laboratories	N CM3762400	191,129
I.S.D.T.	Small Business Administration	N CM3760900	128,829
A.P.	Southern Animal Farms	N CM5772700	32,305
A.P.	Southern Animal Farms	N CM9724500	4,800
R.R.P.	SRI International	N CM4761100	210,934

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE I ACQUISITION OF MATERIALS:		
D.T.P.	Starks Associates, Inc.	N CM4758800	627,257
D.T.P.	Starks Associates, Inc.	N CM4760800	528,026
D.T.P.	State University of New York	N CM2757000	190,797
A.P.	Taconic Farms	N CM3022600	225
A.P.	Taconic Farms	N CM5773000	14,226
A.P.	Texas A & M Research Foundation	N CM3753600	2,691
D.T.P.	VSE Corporation	N CM5765400	191,999
D.T.P.	Warner Lambert	N CM3761400	496,410
	TOTAL		$8,011,385

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
D.T.P.	Arizona, University of	N CM5766200	258,781
D.T.P.	Battelle Memorial Institute	N CM4764800	456,719
D.T.P.	Biotech Research Laboratories, Inc.	N CM3755800	132,855
D.T.P.	Bristol Laboratories	N CM3755600	64,334
D.T.P.	California, University of	N CM5771000	187,079
A.P.	Charles River Breeding Labs.	N CM3762600	1,451,950
A.P.	Charles River Breeding Labs.	N CM3752600	52,500
A.P.	Charles River Breeding Labs.	N CM5765300	97,788
A.P.	Charles River Breeding Labs.	N CM8721200	10,400
A.P.	Charles River Breeding Labs.	N CM9722900	36,745
D.T.P.	Fein Marquart Assoc.	N CM4768300	65,758
A.P.	Harlan Sprague Dawley, Inc.	N CM2391100	750,000
B.R.M.P.	Harlan Sprague Dawley, Inc.	N CM2391100	140,000
A.P.	Harlan Sprague Dawley, Inc.	N CM3762300	438,564
A.P.	Harlan Sprague Dawley, Inc.	N CM5772800	132,638
D.T.P.	IIT Research Institute	N CM4756700	408,923
D.T.P.	Institute of Microbial Chemistry	N CM4759300	130,000
A.P.	Leo Goodwin Inst. for Cancer Research	N CM3762500	192,000
B.R.M.P.	Lymphokine Screen	RFP57717000	150,000
D.T.P.	Mason Research Institute/EG & G	N CM4764700	331,068
D.T.P.	Mayo Foundation	N CM5771100	216,015
A.P.	Microbiological Associates	N CM9728700	192,896
A.P.	Missouri, University of	N CM2753400	44,076
A.P.	Missouri, University of	N CM8715700	95,237
R.R.P.	Northern California Cancer Program	N CM3757800	247,984
A.P.	Northwestern University	N CM1736300	70,608
A.P.	Papanicolaou Cancer Research Institute	N CM8723000	40,960
B.R.M.P.	Preclinical Assessment of MoAb	RFP57719000	500,000
A.P.	Program Resources, Inc.	N C02391000	206,880
B.R.M.P.	Program Resources, Inc.	N C02391000	137,100
A.P.	Simonsen Laboratories	N CM3762400	527,881
A.P.	Simonsen Laboratories	N CM5772900	213,243
A.P.	Southern Animal Farms	N CM5772700	88,839
A.P.	Southern Animal Farms	N CM9724500	13,200
D.T.P.	Southern Research Institute	N CM3755200	157,506
D.T.P.	Southern Research Institute	N CM4764600	538,223
R.R.P.	SRI International	N CM4761100	210,934

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:		
A.P.	Taconic Farms	N CM5773000	39,121
A.P.	Texas A & M Research Foundation	N CM3753600	29,602
D.T.P.	VSE Corporation	N CM5765400	576,000
D.T.P.	Warner Lambert	N CM3761400	87,602
		TOTAL	$9,722,009

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II FORMULATION:		
D.T.P.	Ben Venue Laboratories, Inc.	N CM2750800	126,994
D.T.P.	Iowa, University of	N CM3756100	69,629
D.T.P.	Iowa, University of	N CM4759401	19,610
D.T.P.	Kansas, University of	N CM3756200	199,548
D.T.P.	Kentucky, University of	N CM3762000	120,446
I.S.D.T	Litton Bionetics, Inc.	N CM4765100	166,469
		TOTAL	$702,696

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
D.T.P.	Battelle Memorial Institute	N CM4764800	205,192
D.T.P.	Biotech Research Laboratories, Inc.	N CM3755800	19,255
D.T.P.	California, University of	N CM3756800	128,833
A.P.	Charles River Breeding Labs.	N CM3752600	52,500
A.P.	Charles River Breeding Labs.	N CM9722900	16,703
D.T.P.	Fein Marquart Associates	N CM4768300	20,233
D.T.P.	Fungal Fermentation	RFPT5769200	250,000
A.P.	Harlan Sprague Dawley, Inc.	N CM3762300	200,698
D.T.P.	IIT Research Institute	N CM4756700	183,719
A.P.	Leo Goodwin Institute for Cancer Research	N CM3762500	39,000
D.T.P.	Mason Research Institute/EG & G	N CM4764700	331,067
B.R.M.P.	Meloy Laboratories, Inc.	N CM4765000	203,612
A.P.	Microbiological Associates	N CM9728700	87,680
A.P.	Missouri, University of	N CM2753400	20,034
A.P.	Missouri, University of	N CM8715700	43,289
A.P.	Murphy Breeding Labs., Inc.	N CM3022900	1,836
A.P.	Northwestern University	N CM3753800	32,095
A.P.	Papanicolaou Cancer Research Institute	N CM8723000	18,619
A.P.	Program Resources Incorporated	C02391002P	517,200
A.P.	Simonsen Laboratories	N CM3762400	191,130
A.P.	Southern Animal Farms	N CM5772700	40,382
A.P.	Southern Animal Farms	N CM9724500	6,000
D.T.P.	Southern Research Institute	N CM4758100	301,725
D.T.P.	Southern Research Institute	N CM4761500	180,177
D.T.P.	Southern Research Institute	N CM4764600	241,810
A.P.	Taconic Farms	N CM5773000	17,783
A.P.	Texas A & M Research Foundation	N CM3753600	18,838
D.T.P.	VSE, Corporation	N CM5765400	192,000
		TOTAL	3,561,410

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1985 AS OF 06/30/85 #	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:		
D.T.P.	Aerojet Strategic Propulsion Co.	N CM4758400	202,115
D.T.P.	Aldrich Chemical Company, Inc.	N CM4758500	102,961
D.T.P.	Ash Stevens, Inc.	N CM4758600	274,000
I.S.D.T.	Biotech Research Laboratory, Inc.	N CM3759000	123,630
D.T.P.	Flow Laboratories, Inc.	N CM2750500	140,036
I.S.D.T.	Litton Bionetics, Inc.	N CM0572400	400,000
I.S.D.T.	Litton Bionetics, Inc.	N CM3757500	230,683
I.S.D.T.	Litton Bionetics, Inc.	N CM3758200	128,824
D.T.P.	Midwest Research Institute	N CM3755500	82,525
D.T.P.	Midwest Research Institute	N CM3763404	41,729
D.T.P.	Monsanto Research Corporation	N CM2751600	58,891
D.T.P.	Pharm-Eco	N CM4758700	166,543
D.T.P.	Program Resources, Inc.	N CO2391000C	1,249,423
A.P.	Program Resources, Inc.	N CO2391001P	189,640
D.T.P.	Raylo Chemicals Limited	N CM3763504	12,092
D.T.P.	Research Triangle Institute	N CM2751500	208,006
D.T.P.	Research Triangle Institute	N CM3761900	65,068
D.T.P.	Research Triangle Institute	N CM3763605	15,321
D.T.P.	SISA, Inc.	N CM3763705	21,117
D.T.P.	SISA, Inc.	N CM3763706	15,493
B.R.M.P.	Sloan-Kettering Institute for Cancer Research	N CM2560000	55,000
D.T.P.	Southern Florida, University of	N CM3763904	13,477
D.T.P.	Southern Florida, University of	N CM3763905	10,975
D.T.P.	Southern Research Institute	N CM3763805	22,562
D.T.P.	Southern Research Institute	N CM3763806	30,564
D.T.P.	Southwest Foundation for Research & Education	N CM3764002	38,411
D.T.P.	Stanford Research Institute	N CM3761800	97,174
D.T.P.	Stanford Research Institute	N CM3764105	15,428
D.T.P.	Stanford Research Institute	N CM3764106	23,868
D.T.P.	Starks Associates, Inc.	N CM3764203	13,674
D.T.P.	Starks Associates, Inc.	N CM3764204	23,873
D.T.P.	Starks Associates, Inc.	N CM3764205	7,935
D.T.P.	Starks Associates, Inc.	N CM3764206	10,256
D.T.P.	Warner Lambert	N CM4758900	182,485
		TOTAL	$ 4,273,779

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1985 AS OF 06/30/85 #	DOLLAR LEVEL
	STAGE III PHARMACOLOGY/TOXICOLOGY:		
D.T.P.	Battelle Memorial Institute	N CM1736500	1,889,989
D.T.P.	Institute Jules Bordet	N CM5764500	150,212
D.T.P.	Master Agreement for Preclinical Pharmacology	RFPT1736400	500,000
D.T.P.	Mayo Foundation	N CM3760103	60,043
D.T.P.	Mayo Foundation	N CM3760104	81,674
A.P.	Texas A&M Research Foundation	N CM3753600	2,691
		TOTAL	$ 2,684,609

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1985 AS OF 06/30/85 #	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	Aerojet Strategic Propulsion Co.	N CM4758400	202,114
D.T.P.	Aldrich Chemical Company, Inc.	N CM4758500	191,212
D.T.P.	Arizona, University of	N CM3762100	127,554
D.T.P.	Ash Stevens, Inc.	N CM4758600	274,000
D.T.P.	Ben Venue Laboratories, Inc.	N CM2750800	1,606,941
D.T.P.	Ben Venue Laboratories, Inc.	N CM5759500	286,539
D.T.P.	Flow Laboratories, Inc.	N CM1739800	325,000
D.T.P.	Flow Laboratories, Inc.	N CM2750500	46,678
D.T.P.	Georgia, University of	N CM2740100	173,058
D.T.P.	Iowa, University of	N CM3756100	278,515
D.T.P.	Iowa, University of	N CM4759401	78,442
D.T.P.	Midwest Research Institute	N CM3755500	330,102
D.T.P.	Monsanto Reserach Corporation	N CM2751600	431,868
D.T.P.	Pharm-Eco	N CM4758700	166,543
D.T.P.	Research Triangle Institute	N CM2751500	52,001
D.T.P.	Research Triangle Institute	N CM3761900	260,274
D.T.P.	Stanford Research Institute	N CM3761800	388,696
D.T.P.	Warner Lambert	N CM4758900	182,484
		TOTAL	$ 5,402,021

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - BIOLOGICAL STUDIES		
B.R.M.P.	Iowa, Univeristy of	N CM3757900	138,115
I.S.D.T.	Litton Bionetics, Inc.	N CM3757500	230,682
I.S.D.T.	Litton Bionetics, Inc.	N CM2561600	579,507
C.O.P.	Litton Bionetics, Inc.	N CM4765700	54,892
C.O.P.	Litton Bionetics, Inc.	N CM4764900	176,035
C.O.P.	Meloy Laboratories, Inc.	N CM5768800	210,691
C.O.P.	Veterans Administration Medical Center	Y CM3025600	37,500
	TOTAL		$ 1,427,422

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1985 AS OF 06/30/85	#	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:			
R.R.P.	Arizona		N CM1752200	122,415
R.R.P.	Energy, Department of-Lawerence Berkeley Lab		Y CM2011000	91,024
C.O.P.	Litton Bionetics, Inc.		N CM4764900	71,902
R.R.P.	Mass. Inst. Tech.		N CM2752500	59,707
R.R.P.	Massachusetts General Hospital		N CM2753200	129,349
R.R.P.	Massachusetts General Hospital		N CM4761600	106,398
R.R.P.	Memorial Hospital for Cancer & Allied Diseases		N CM4769500	127,780
C.O.P.	Orkand Corporation		N CM3601000	345,975
R.R.P.	Pennsylvania, University of		N CM2752900	59,794
R.R.P.	Pennsylvania, University of		N CM4769700	112,426
R.R.P.	Sloan-Kettering		N CM3756500	366,006
D.T.P.	Southern Research Institute		N CM4758000	611,630
D.T.P.	Southern Research Institute		N CM3755200	128,868
R.R.P.	Stanford University		N CM1748000	80,421
R.R.P.	Texas, University of,		N CM2753100	101,100
R.R.P.	Texas University		N CM1752400	93,686
R.R.P.	Utah University		N CM1752300	74,250
R.R.P.	Washington, University of		N CM4769600	116,371
		TOTAL		$ 2,799,102

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1985 AS OF 06/30/85 #	DOLLAR LEVEL
	PROGRAM MANAGEMENT:		
D.T.P.	Battelle Memorial Institute	N CM1736500	809,995
C.T.E.P.	Emmes Corporation	N CM1737100	243,301
C.T.E.P.	Information Management Services, Inc.	N CM2751000	300,000
O.D.	JWK International Corporation	N CM2560200	50,000
B.R.M.P.	Koba, Inc.	N CM4766100	369,137
A.P.	National Academy of Sciences	N CM5764400	34,000
D.T.P.	ORI, Inc.	N CM3759100	101,458
C.T.E.P.	Social & Scientific Systems, Inc.	N CM3757600	939,918
C.T.E.P.	Social & Scientific Systems, Inc.	N CM2560600	90,000
O.D.	Technical Resources, Inc.	N CM5765800	179,348
		TOTAL	$ 3,117,157

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1985 AS OF 06/30/85 #	DOLLAR LEVEL
	PHASE I CLINICAL TRIALS:		
R.R.P.	Arizona, University of	N CM1752200	122,416
R.R.P.	Howard University	N CM2754300	0
C.T.E.P.	Johns Hopkins University	N CM2750900	70,333
C.T.E.P.	Johns Hopkins University	N CM5773800	133,731
C.T.E.P.	Maryland, University of	N CM2754100	78,120
C.T.E.P.	Maryland, University of	N CM5773400	145,030
R.R.P.	Massachusetts General Hospital	N CM1748100	0
R.R.P.	Massachusetts Institute of Technology	N CM2752500	59,854
R.R.P.	Mayo Foundation	N CM2752800	59,005
C.T.E.P.	Mayo Foundation	N CM2754800	94,270
C.T.E.P.	Mayo Foundation	N CM5773300	144,780
C.T.E.P.	Memorial Hospital for Cancer & Allied Diseases	N CM2754600	76,974
C.T.E.P.	Memorial Hospital for Cancer & Allied Diseases	N CM5773200	172,096
C.T.E.P.	Ohio State University Research Foundation	N CM2754000	65,105
C.T.E.P.	Ohio State University Research Foundation	N CM4762200	121,535
C.T.E.P.	Ohio State University Research Foundation	N CM5773600	126,347
B.R.M.P.	Phase I Clinical Trials	RFP37613000	600,000
R.R.P.	Stanford University	N CM1748000	80,422
R.R.P.	Texas, University of	N CM1752400	93,687
C.T.E.P.	Texas, University of	N CM5773700	157,802
C.T.E.P.	Texas, University of, Health Science Center	N CM2754200	72,027
C.T.E.P.	Texas, University of, System Cancer CTR/MDA	N CM2755000	53,621
C.T.E.P.	Texas, Universtiy of, System Cancer CTR/MDA	N CM57739	134,044
R.R.P.	Utah, University of	N CM1752300	74,250
C.T.E.P.	Wayne State University	N CM2755100	76,351
C.T.E.P.	Wisconsin, Univeristy of	N CM2754900	96,992
C.T.E.P.	Wisconsin, University of	N CM5773500	126,566
	TOTAL		$3,035,358

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS		
R.R.P.	Health Research Institute	N CM9731100	43,699
R.R.P.	Howard University	N CM2754300	0
C.T.E.P.	Maryland, University of	N CM5773400	98,590
R.R.P.	Massachusetts General Hospital	N CM1748100	0
R.R.P.	Mayo Foundation	N CM2752800	59,005
C.T.E.P.	Mayo Foundation	N CM5773300	85,619
C.T.E.P.	Memorial Hospital for Cancer & Allied Diseases	N CM0733700	70,011
C.T.E.P.	Memorial Hospital for Cancer & Allied Diseases	N CM5773200	103,622
C.T.E.P.	Michigan, University of	N CM0740500	171,562
B.R.M.P.	Phase II Clinical Trials	RFP37613000	597,000
R.R.P.	Southern California, University of	N CM2748300	46,091
C.T.E.P.	Texas, University of, System Cancer CTR/MDA	N CM5773900	90,795
C.T.E.P.	Wayne State University	N CM0740400	67,500
		TOTAL	1,433,494

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS		
C.T.E.P.	Maryland, University of	N CM5773400	10,955
C.T.E.P.	Mayo Foundation	N CM5773300	9,513
C.T.E.P.	Memorial Hospital for Cancer & Allied Diseases	N CM0733700	7,779
C.T.E.P.	Memorial Hospital for Cancer & Allied Diseases	N CM5773200	11,514
C.T.E.P.	Michigan, University of	N CM0740500	19,062
C.T.E.P.	Pan American Health Organization	N CM2739100	388,764
C.T.E.P.	Texas, University of, System Cancer CTR/MDA	N CM5773900	10,078
C.T.E.P.	Wayne State University	N CM0740400	7,500
		TOTAL	465,165

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 06/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS - OTHER RESEARCH		
R.R.P.	Bowman Gray School of Medicine	N CM4756400	197,741
R.R.P.	California, University of	N CM9731500	1,279,150
R.R.P.	California, University of (SF)	N CM4768400	243,844
R.R.P.	Cleveland Clinic Foundation	N CM4768500	286,548
R.R.P.	Duke University	N CM4768600	210,891
R.R.P.	Fox Chase Cancer Center	N CM9731400	1,277,210
C.T.E.P.	Istituto Nazionale Tumori	N CM0733800	145,000
R.R.P.	Massachusetts General	N CM4768700	259,089
R.R.P.	Washington, University of	N CM9728200	958,821
		Total	4,858,294

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 6/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH		
R.R.P.	Allegheny-Singer Research Corporation	N CM3751200	367,320
R.R.P.	Bureau of Radiological Health, FDA	Y CM2010700	80,700
R.R.P.	Development of Dosimitry Standards for Neutron Therapy	RFPT8500300	120,000
C.T.E.P.	Emmes Corporation	N CM1737100	243,302
C.T.E.P.	Emmes Corporation	N CM8719300	277,597
C.T.E.P.	Information Management Services, Inc.	N CP3100900	30,000
C.T.E.P.	Information Management Services, Inc.	N CM2751000	300,000
O.D.	Japanese Foundation for Cancer Research	N CM3601100	19,000
C.T.E.P.	Theradex Systems, Inc.	N CM3755300	1,026,923
		Total	2,464,842

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY FOR FISCAL YEAR 1985
AS OF 6/30/85

AREA	CONTRACT	#	DOLLAR LEVEL
	STATISTICS, DATA PROCESSING, AND OTHER CLINICAL TRIAL RESOURCES		
D.T.P.	Chemical Abstract Services	N CM4770900	94,217
C.O.P.	Litton Bionetics, Inc.	N CM4765700	6,099
D.T.P.	Maintenance of NCI/DIS	RFPT4768900	90,000
C.O.P.	Meloy Laboratories, Inc.	N CM5768800	23,410
R.R.P.	Orkand Corporation	N CM3757200	0
		Total	213,726

TABLE III

DESCRIPTION OF CONTRACTS

IN THE

DIVISION OF CANCER TREATMENT

AEROJET STRATEGIC PROPULSION COMPANY (NO1-CM4-7584)

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-CM2-7571)

This project is one of the 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) improved bio-availability by increasing aqueous solubility; (b) increased compound stability; and (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.

ALABAMA, UNIVERSITY OF (NO1-CM3-7631)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

ALDRICH CHEMICAL COMPANY, INC. (NO1-CM4-7585)

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.

ALLEGHENY-SINGER RESEARCH CORPORATION (NO1-CM3-7512)

This contract is expected to develop the criteria, guidelines and procedures for the proper use of the equipment representing the major heat generating modalities (radiofrequency, microwave, and ultrasound) and the ancillary equipment necessary for the treatment of cancer with heat. The criteria and guidelines developed will be utilized to initiate a quality assurance and assessment program in hyperthermia. The contractor is expected to implement and conduct such a program in the latter years of the contract.

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 National Center for Devices and Radiological 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.

ARIZONA, UNIVERSITY OF (NO1-CM3-7621)

Difficult dosage form development projects are assigned to this contractor for evaluation. The contractor has particular expertise with cosolvent approaches to improve drug solubility. Several novel solvents are being evaluated in an attempt to improve the options available to formulate poorly water soluble and unstable compounds. This contractor is also responsible for the preparation of pilot batches and chemical analysis of these novel formulations.

ARIZONA, UNIVERSITY OF (NO1-CM5-7662)

This is one of three contracts devoted to the application of a human tumor colony forming assay (HTCFA) to drug screening. Contract efforts have focused on screening compounds inactive in the standard in vivo pre-screen (murine P388 leukemia) to establish the potential of the HTCFA to identify compounds not detected by the in vivo screen. Materials active against fresh human tumor specimens are also tested in a P388 cell line colony forming assay, developed under this project for direct comparison with the HTCFA. Screening has been initiated on more than 300 compounds and several have been identified as anti-tumor drug leads. Activity in the HTCFA, P388 colony forming assay, in vivo tumor panel, and degree of structural novelty, are used to determine which are recommended to the Decision Network Committee for development to clinical trial. To date three compounds have been accepted into the formal drug development program.

ARMED FORCES INSTITUTE OF PATHOLOGY (YO1-CM3-0118)

This Interagency Agreement provided 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. This Interagency Agreement expired January, 1985.

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 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. The contractor is currently working on a task to characterize the preclinical pharmacology of Deoxyspergualin, NSC 356894.

ASH STEVENS, INC. (NO1-CM4-7586)

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-CM1-7365)

This service type Prime Contract with Battelle Memorial Institute is for supervision of subcontractors carrying out the toxicologic evaluation of potential oncolytic agents, biologic response modifiers, 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, anomaly detection, scheduling of studies, and financial management.

BATTELLE MEMORIAL INSTITUTE (NO1-CM4-7648)

This contract is for the in vivo screening of new materials in the P388 leukemia pre-screen and for the secondary testing of materials of interest to the Developmental Therapeutics Program. The secondary testing may consist of testing in both a panel of murine tumor models and a human xenograft tumor model. Testing is conducted at a level of approximately 20,000 L1210 equivalents per year. Special studies for the detailed evaluation of compounds of Program interest, as well as studies with new tumor panel systems, are conducted upon the request of the Project Officer.

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

This resource contract provides for the development and production of parenteral clinical dosage forms of anticancer agents. The contractor has the capacity for preparing production batches of 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.

BEN VENUE LABORATORIES, INC. (NO1-CM5-7595)

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 shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

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.

BOWMAN GRAY SCHOOL OF MEDICINE (NO1-CM4-7564)

This is one of five contracts awarded in May 1984 (a) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of 1) musculoskeletal tumors, 2) cervical myelopathies, 3) uterine neoplasms, and 4) liver metastases. They have also provided coordination for the CWG of the development of a lung cancer protocol. Patient imaging has begun in each of these studies. A breast disease protocol has also been prepared for adoption in year 02.

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

This fermentation contract is designed primarily to discover novel antitumor agents from microbial fermentations. This contract includes: (1) Isolation of microbes and screening these for unique biological activities. The isolations and screening are done mostly at the Bristol Myers Japanese facility, Bristol Myers Research Institute - Tokyo (BMRIT); (2) Preparation of fermentation beers of the selected microbes; (3) Development and use of in vitro pre-screens to evaluate the fermentations; (4) Development of in vitro assays to assist in quickly isolating the anticancer agents; (5) Dereplication of the materials to determine novelty; (6) Chemical isolation and characterization of the active components; and (7) Production of large quantities of new agents for thorough evaluation in DCT screens. This contract will terminate in FY 1986.

CALIFORNIA, UNIVERSITY OF (NO1-CMO-7439)

On this contract, Phase I/II clinical evaluations of Wellferon are being conducted in patients with advanced breast cancer and multiple myeloma. Although the above trials have now been completed, an ongoing trial of natural interferon in hairy cell leukemia is continuing. This contract will expired June, 1985.

CALIFORNIA, UNIVERSITY OF (NO1-CM3-7568)

Project objectives are (1) to determine the extent to which in vitro clonogenic assays, using transplantable human tumor/athymic mouse xenografts as the source of tumor cells are capable of identifying drugs with activity against the same tumors in vivo and (2) to develop disease-oriented preclinical screens that permit expression of drug activity as response rate. This project ended August, 1985.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-7672)

Under this contract, a Phase I clinical trial is to be conducted with a monoclonal immunoconjugate. Pharmacokinetics, clinical toxicity, and biologic response will be monitored. This trial is scheduled to begin in late spring. The contract will expire December, 1985.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-7684)

This is one of five contracts awarded in May 1984 (a) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of: 1) musculoskeletal tumors, 2) cervical myelopathies, 3) uterine neoplasms, 4) liver metastases, and 5) brain neoplasms. They have also provided coordination for the CWG of the development of the uterine neoplasms protocol. Patient imaging has begun in each of these studies. A cardiovascular disease protocol has also been prepared for adoption in year 02.

CALIFORNIA, UNIVERSITY OF (NO1-CM5-7710)

This is one of three contracts devoted to the application of a human tumor colony forming assay (HTCFA) to drug screening. Contract efforts have focused on screening compounds inactive in the standard in vivo pre-screen (murine P388 leukemia) to establish the potential of the HTCFA to identify compounds not detected by the in vivo screen. Materials active against fresh human tumor specimens are also tested in a P388 cell line colony forming assay, developed under this project for direct comparison with the HTCFA. Screening has been initiated on more than 300 compounds and several have been identified as anti-tumor drug leads. Activity in the HTCFA, P388 colony forming assay, in vivo tumor panel, and degree of structural novelty, are used to determine which are recommended to the Decision Network Committee for development to clinical trial. To date three compounds have been accepted into the formal drug development program.

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, and was scheduled for completion in April, 1984. The Cyclotron Corporation, which is manufacturing the cyclotron and neutron therapy system, filed for bankruptcy under Chapter 11 of the Bankruptcy Code. The neutron therapy system, which was approximately 90% complete, has been moved to the VA Hospital facilities for final assembly and testing. Patient treatments are expected to begin in November, 1985.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM3-7526)

This contract provides for the rederivation of approximately 16 mouse and rat strains and two guinea pig strains on an annual basis. Rederived strains will be distributed to genetic centers for expansion and replacement of producing strains.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM3-7626)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for hybrid production and the many NCI research activities.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM5-7653)

This Rodent Production Center contract, located in North Carolina, produces hybrid mice and nude mice in a maximum barrier environment.

CHARLES RIVER BREEDING LABORATORIES (NO1-CM8-7212)

This contract provides for the continual monitoring of the 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-CM9-7229)

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. This contract terminated January, 1985.

CHEMICAL ABSTRACTS SERVICE (NO1-CM4-7709)

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. This contract was phased out April 15, 1985; the system, which is now ten years old, being replaced by a newer one.

CLEVELAND CLINIC (NO1-CM4-7685)

This is one of five contracts awarded in May, 1984 (a) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the non-invasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of 1) musculoskeletal tumors, 2) cervical myelopathies, 3) uterine neoplasms, 4) liver metastases, and 5) brain neoplasms. They have also provided coordination for the CWG of the development of the cervical myelopathies protocol. Patient imaging has begun in each of these studies.

DUKE UNIVERSITY (NO1-CMO-7436)

This contract is conducting Phase I/II clinical trials of recombinant gamma interferon in the treatment of patients with disseminated malignant disease. Sixteen patients have been accrued to the initial step of this study, which is now completed. Fever has been the major toxicity. The contract has been extended without additional cost through June 30, 1985 to permit an additional study of the effects of repeated administration of gamma interferon at a single dose to be performed.

DUKE UNIVERSITY (NO1-CM4-7686)

This is one of five contracts awarded in May, 1984 (a) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the non-invasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of 1) musculoskeletal tumors, 2) cervical myelopathies, 3) uterine neoplasms, 4) liver metastases, 5) brain neoplasms, and 6) lung cancer. They have also provided coordination for the CWG of the development of the brain neoplasms protocol. Patient imaging has begun in each of these studies.

DYNAMAC CORPORATION (NO1-CM3-7563)

The objective of this project is to develop and maintain a systematic literature surveillance effort to identify published compounds which warrent 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.

EG&G MASON RESEARCH INSTITUTE (NO1-CM4-7647)

As the result of budgetary restraints, this contract was reduced during the current contract period to a level of 21,500 L1210 equivalents (an annualized level of 19,000 equivalents). The contract provides for in vivo testing in the P388 leukemia pre-screen, for the evaluation of materials in specified tumor panel models, for detailed evaluations as requested by members of the NCI staff, and for evaluations of tumor models as directed by the Project Officer. In addition to the P388, other tumor systems currently being utilized include the murine L1210, M5076 sarcoma, and B16 (being phased out). Xenograft models include the colon, breast and lung sub-renal capsule models, the LOX amelanotic melanoma and the HL-60 which is used for special testing of differentiating agents.

EMMES CORPORATION (NO1-CM1-7371)

This contract provides operations office support for the Gastrointestinal Tumor Study Group and the Intergroup Testicular Cancer Studies. 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. During this period the Contractor has also provided research and information management assistance to the CTEP in support of its mission of coordination of ongoing and planned clinical trials.

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.

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.

ENGLE LABORATORY ANIMALS (NO1-CM3-0225)

This contract furnishes approximately 104,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated January, 1985.

FEIN-MARQUART ASSOCIATES, INC. (NO1-CM4-7683)

Under this contract, work has continued for the past year upon the development of the NCI Drug Information System (DIS). The major subsystems of the DIS, the Chemistry, Inventory and Shipping modules have all been installed, tested, and released for use. Trouble shooting in the first two of these is essentially complete. Some problems remain in the Shipping module and are currently being addressed. Meanwhile, the Biology module is being readied for installation. The previous computer system, which the DIS is replacing, has now been frozen and all new data have been flowing into the DIS since 3/85. The old contract has been terminated. The troublesome problem of documentation has been addressed addressed and a major documentation effort, with a high priority, has begun. This should be largely complete by 9/85 when the contract ends.

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

This resource contract provides the Division of Cancer Treatment with storage and distribution capabilities for the 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 many activities of the Developmental Therapeutics Program.

FOX CHASE CANCER CENTER (NO1-CMO-7432)

This contract is now being conducted by Jefferson Medical Center, Pennsylvania, under a subcontract to the Fox Chase Cancer Center. It was designed to study the Phase I effects of decreasing the number of circulating suppressor T cells in patients with disseminated malignancy by the infusion of anti-T suppressor cell antibody (anti-Leu 2a, Becton-Dickinson). Doses of 1, 5, 25, and 100mg have been administered by infusions lasting from 2 hours to 24 hours. Sixteen patients have been accrued to the first step of this protocol. Pharmacokinetic data and immunologic data are being evaluated at this time. A second step of the study will evaluate the effects of repetitive administration of the optimal biologic dose. Toxicity has included fever and shaking chills. The contract was extended through June, 1985.

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 first DT generator tube was shipped to Philadelphia in March, 1982. Unexpected problems with a series of tubes caused interruptions during 1982, 1983, and 1984. Patient treatments began in January, 1984, continued for about two months and then were suspended for about a year, resuming in February, 1985.

FRANKLIN RESEARCH INSTITUTE (NO1-CM3-7632)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CM4-7668)

On this contract, a Phase I clinical trial is to be conducted with a monoclonal immunoconjugate directed against malignant melanoma. Pharmacokinetics, clinical toxicity, and biologic response will be monitored. This trial is scheduled to begin in late spring. The contract will expire December, 1985.

GEORGETOWN UNIVERSITY (NO1-CMO-7437)

On this contract Phase II clinical trials of Wellferon in patients with metastatic melanoma. In the first step of this study, three different schedules were compared in a total of 32 patients. At present four patients have shown a complete or partial response. Responses were seen in both the alternate day and daily schedule, but not on the weekly schedule. The most significant toxicity was observed in one patient, who developed a lupus-like reaction while receiving the alpha interferon. This contract has received supplemental funding to conduct an additional clinical study in patients with malignant melanoma. The contract expiree August, 1985.

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 evaluation 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 TECHNICAL RESEARCH CORPORATION (NO1-CM2-7517)

This project is one of the 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; and (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/SPRAGUE-DAWLEY, INC. (NO1-CM2-3911)

This contract operates the Animal Production Area at the Frederick Cancer Research Facility (FCRF). 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 the 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 the FCRF. Animals are also sent from FCRF to other NCI funded research activities.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM3-7623)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for the many NCI research activities.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM5-7728)

This Rodent Production Center contract produces CD2F1 hybrid mice under maximum barrier conditions.

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 nonderivatized 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 ongoing to couple adriamycin, methotrexate, ricin A chain, Yttrium-90, Indium-111 and Iodine-131 to three 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. The contractor has been supplying T101 conjugated to indium and iodine for biodistribution and imaging studies and is scaling up to provide T101 and 9.2.27 drug conjugates for clinical studies. This contract expired September, 1985, and is being recompeted.

IIT RESEARCH INSTITUTE (NO1-CM4-7567)

This contract, awarded as a result of the recompetition of the in vivo screening program, has a current level of testing in mice of potential anticancer agents of 19,000 L1210 equivalent tests per year, having been reduced during FY-85 due to budgetary restrictions. 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 prescreen 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 M5076 sarcoma and L1210 leukemia. Xenograft models include the breast sub-renal capsule model and the LOX amelanotic melanoma.

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 of these compounds which may be related to cancer. Reports of biological activity of plant and animal extracts are also provided. This is a key contract for acquisition of new agents for the DTP screening program. This contract is currently being recompeted.

ILLINOIS CANCER COUNCIL (NO1-CM4-7667)

This contract will evaluate the clinical effects of a murine anti-T-cell monoclonal immunoconjugate, T101-I^{131} in a Phase I clinical trial. Pharmacokinetics, relevant biologic responses, and clinical toxicity will be monitored. In addition, localization in tumor and normal tissues will be compared. The trial began in mid-April of this year.

This contract will also conduct a Phase I trial evaluating the clinical toxicity and effects of recombinant interleukin-2 and natural interleukin-2. Five patients will be initiated at each starting dose level and be escalated through four additional doses. This trial began in early May of this year. This contract will expire December, 1985.

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.

The contract will be extended noncompetitively during Fiscal Year 1985. The project will be recompeted in Fiscal Year 1986.

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CP3-1009)

An interdivisional transfer of funds to DCE, Biometry Branch, to support statistical services for the Intergroup Testicular Studies. Monies support extramurally contracted data management with IMS.

INSTITUT JULES BORDET (NO1-CM5-7645) (FORMERLY NO1-CMO-7350)

Materials collected in Western 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-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. This contract expired June, 1985.

INSTITUTE OF MICROBIAL CHEMISTRY (NO1-CM4-7593)

The major objective of this contract is the isolation of new antitumor agents from fermentations of marine and terrestrial microorganisms. 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. and another, deoxyspergualin, will enter Phase I trials shortly.

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

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 set a standard for therapeutic studies in this country and around the world. The Istituto has shown an improved overall survival for premenopausal patients treated with CMF, and that twelve months of CMF is no more effective than six months. More recent analyses indicate that survival of patients following relapse is unaffected by whether or not they received adjuvant chemotherapy, and that estrogen receptor status is an independent prognostic indicator for pre-menopausal women. In addition, they have developed an extensive data base on the utility of labelling index, which appears to be a more powerful prognosticator than hormone receptor status.

IOWA, UNIVERSITY OF (NO1-CM3-7561)

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 developed and produced primarily sterile freeze-dried injectable products under this contract. However, this contractor has the capability to produce a wide variety of pharmaceutical dosage forms. The contractor is also responsible for completing all required analytical and safety tests on each lot of drug. All products are packaged, labeled, and shipped to the National Cancer Institute for redistribution to clinical investigators.

IOWA, UNIVERSITY OF (NO1-CM3-7579)

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 in common pharmaceutical vehicles. Studies include determination of amino acid composition, molecular weight, isoelectric point, terminal sequence and development of suitable immunological measurement (radioimmunoassays, etc.) and suitable biological assays for qualitative and quantitative evaluations. In the past year this contractor has analyzed and characterized several lots of naturally occuring alpha, beta and gamma interferons for activity and purity, analyzed several natural and recombinant IL-2 preparations, natural B-cell growth factor preparations, purified the tetrapeptide tuftsin for preclinical screening, examined a murine tumor necrosis factor preparation for purity and provided near homogeneous material for monoclonal antibody production.

IOWA, UNIVERSITY OF (NO1-CM4-7594)

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 and in a manner to protect personnel from exposure to potentially toxic agents. The contractor is also responsible for all required quality control tests on each product prepared. These dosage forms are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM3-6011)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo to provide up-to-date information services in support of our cancer treatment program, both preclinical and clincial. This is a cost-sharing Contract and is strongly supported by the Japanese Foundation for Cancer Research and the Japanese Society for the Promotion of Science.

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. The Contractor is presently conducting Phase I studies with Spiromustine and Taxol and has just started a new trial with HMBA. This contract expired July, 1985.

JWK INTERNATIONAL CORPORATION (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 logistial 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, and expired March, 1985. This contract was recompeted during FY 1985 and was awarded to another firm.

KANSAS, UNIVERSITY OF (NO1-CM3-7562)

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.

KENTUCKY RESEARCH FOUNDATION, UNIVERSITY OF (NO1-CM3-7620)

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.

KING ANIMAL LABORATORIES, INC. (NO1-CM3-0228)

This contract furnishes approximately 104,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated January, 1985.

KOBA ASSOCIATES (NO1-CM4-7661)

This contract provides technical support for the collection, review and compilation of available information on biological response modifiers for possible development through preclinical and clinical trials. The contractor has responsibility for obtaining pertinent information from sources in industry, institutes universities as well as through literature review. Information and references obtained are submitted to the program in the form of review articles and one page synopses. Information gathered is stored in a computer data bank. Each year the program specifies about 100 compounds for the contractors to research. The contractor has provided about 50 one page synopses and 30 five page reviews.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CM3-7625)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for the many NCI research activities.

LITTON BIONETICS, INC. (NO1-CMO-5724)

This contract provides animal facilities to house small and large animals, including rats, cats, rabbits, goats and monkeys. The animals are used for the preparation of antibodies as well as for inoculation of tumor cells and virus preparations for tumorigenicity testing.

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, INC. (NO1-CM3-7575)

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; and (4) to test sera from patients with T cell leukemia for antibodies to human type C RNA tumor virus (HTLV).

LITTON BIONETICS, INC. (NO1-CM3-7582)

This contract provides supportive services in the supply of small quantities of tissue culture cells, radiolabeled DNA and RNA probes.

LITTON BIONETICS, INC. (NO1-CM4-7651)

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

LITTON BIONETICS, INC. (NO1-CM4-7657)

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. This contract terminated April, 1985.

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. The Contractor is presently conducting Phase I studies with Spiromustine and Menogaril and has started a new trial with HMBA. This contract expired July, 1985.

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 GENERAL HOSPITAL (NO1-CM4-7616)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM4-7687)

This is one of five contracts awarded in May, 1984 (a) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of 1) musculoskeletal tumors, 2) cervical myelopathies, 3) uterine neoplasms, 4) liver metastases, and 5) brain neoplasms. They have also provided coordination for the CWG of the development of the musculoskeletal tumor and liver metastases protocols. Patient imaging has begun in each of these studies. This contractor has been influential in guiding the statistical design of several of the protocols.

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 National Center for Devices and Radiological 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.

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-CM2-7548)

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 is presently conducting Phase I studies with Tiazofurin, Hexamethylmelamine IV, a combination trial with PALA/5-FU/TdR, and a new study will begin shortly with BIDA. This contract expired July, 1985.

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. The contractor is currently working on tasks to characterize the preclinical pharmacology of Pibenzimol, NSC 322921, Phyllanthoside, NSC 328426 and Teroxirone, NSC 296934.

MAYO FOUNDATION (NO1-CM5-7711)

This is one of three contracts devoted to the application of a human tumor colony forming assay (HTCFA) to drug screening. Contract efforts have focused on screening compounds inactive in the standard in vivo pre-screen (murine P388 leukemia) to establish the potential of the HTCFA to identify compounds not detected by the in vivo screen. Materials active against fresh human tumor specimens are also tested in a P388 cell line colony forming assay, developed under this project for direct comparison with the HTCFA. Screening has been initiated on more than 300 compounds and several have been identified as anti-tumor drug leads. Activity in the HTCFA, P388 colony forming assay, in vivo tumor panel, and degree of structural novelty, are used to determine which are recommended to the Decision Network Committee for development to clinical trial. To date three compounds have been accepted into the formal drug development program.

MELOY LABORATORIES, INC. (NO1-CM4-7650)

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 60 different biologics. In the past year the contractor has performed general safety, pyrogenicity, purity and other relevant testing on several lots of monoclonal antibody preparations for use in clincial trials from within the program, as well as preparations submitted from other NCI and NIH scientists. The contract also provides for ascites production and purification of monoclonal antibody and has produced monoclonals specific for melanoma, colon, and breast cancers.

MELOY LABORATORIES, INC. (NO1-CM5-7688)

This contract supports Surgery Branch research by providing appropriate amounts of human and murine Interleukin-2 to conduct experiments. In addition, murine and human lymphokine activated killer cells are generated for therapeutic experiments. This research is directed toward developing new adoptive immuno-therapies for the treatment of cancer using specifically sensitized lymphoid cells expanded in Interleukin-2 or using Interleukin-2 directly as an immune adjuvant.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CMO-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 NCI and the Principal Investigator. This contract expired June, 1985.

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

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. The Contractor is presently conducting Phase I studies with Fluoro-Ara-AMP in pediatric patients, a combination Phase I trial with PALA + MTX + 5-FU, Tiazofurin, and Trimetrexate. This contract expired July, 1985.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-7695)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

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 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 NCI and the Principal Investigator. Patient accrual continued through July, 1985, with follow-up ending in February, 1986.

MICHIGAN TECHNOLOGICAL UNIVERSITY (NO1-CM3-7633)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

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; 2) to test experimental tumors (animal and human) for viral contaminants; 3) to perform ELISA tests annually for the detection of mouse hepatitis virus (MHV); and 4) to produce vaccinia virus which is used for immunizing mice against infectious ectromelia.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-7555)

Midwest Research Institute is one of the three 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.

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. The contractor is currently working on a task to characterize the preclinical pharmacology of Caracemide, NSC 253272 and Melphalan, NSC 8806.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-7634)

This is one of the 12 master contractors 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 re-synthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

MISSOURI, UNIVERSITY OF (NO1-CM2-7534)

This contract monitors the animal production and screening laboratory by testing for the presence of Salmonella and Pseudomonas. Samples are received on a scheduled basis from the animal producers and screening laboratories and approximately 9,000 fecal samples are tested per year.

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 multi-kilogram range, requiring pilot plant facilities.

MOUNT SINAI MEDICAL CENTER (NO1-AI2-2669)

An inter-institutional transfer of funds to the NIAID 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. NCI support for this contract will terminate in Fiscal Year 1985. NIAID will continue to support this project.

MURPHY BREEDING LABORATORIES (NO1-CM3-0229)

This contract furnishes approximately 104,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated January, 1985.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-7644)

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 commmittees whose membership represent commercial animal production colonies, governmental and academic institutions, and non-profit research institutions.

NATIONAL CENTER FOR DEVICES AND 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 Physical Sciences, National Center for Devices and Radiological Health, Food and Drug Administration, 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.

NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CM3-7578)

The capability for evaluating chemical compounds for radiation sensitizing and/or radiation protective 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 or radioprotective characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiosensitization using mammalian cell cultures. Potential radiosensitizing 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 radioprotective compounds tested 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 radiosensitizers and radioprotectors or leads in developing new types (classes) of radiation modifying compounds.

NORTHWESTERN UNIVERSITY (NO1-CM3-7538)

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.

OHIO STATE UNIVERSITY (NO1-CMO-7442)

This Phase I/II clinical contract trial, evaluating both beta and gamma recombinant interferon in patients with renal cell carcinoma is continuing. Seven patients have been studied with gamma interferon. In four patients, the maximally tolerated dose (MTD) was 12 million units; however, in another three patients, the MTD ranged from 30-70 million units. Three additional patients have received beta interferon. This contract expired June, 1985.

OHIO STATE UNIVERSITY (NO1-CM4-7622)

The principal objective of this project is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease during Phase I studies to help establish the most effective dosage schedule. Two new or established cancer chemotherapeutic agents are evaluated annually. The studies involve approximately 30 patients per drug. During this period the Contractor evaluated Pibenzimol.

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

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 is presently conducting Phase I studies with Acodazole and 2-FAMP, and has started two new trials with Pibenzimol and BIDA. This contract will expired July, 1985.

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 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. The contractor is currently working on a task to characterize the preclinical pharmacology of Pibenzimol, NSC 322921, Aphidicolin Glycinate, NSC 303812 and Merbarone, NSC 336628.

ORI, INC. (NO1-CM3-7591)

This contract is intended to provide quick response programming support, to enable the ITB to deal with the unanticipated problems or urgent requirements that are encountered in the operation of the ITB chemical and biological information system. Tasks have included, thus far, the transfer of the Hodes model to the DIS Pre-Registry subsystem, and Systems Analysis support for the DIS graphic subsystem (laser printer) and the DIS data entry module.

ORKAND CORPORATION (NO1-CM3-6010)

This contract supports the Clinical Oncology Program of the Division of Cancer Treatment with computer programming 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 Clinical Oncology Program.

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 data in clinical oncology. Lesser numbers of Phase III studies are left at this point.

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 or 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.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM4-7697)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

PHARM-ECO LABORATORIES, INC. (NO1-CM4-7587)

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. Materials worked on include pancratistatin, phyllanthoside, and taxol. Process development on these plant products has been completed. This contract is currently being recompeted.

PROGRAM RESOURCES, INC. (NO1-CO2-3910)

This contract is located at the Frederick Cancer Research Facility in Frederick, Maryland. The effort is divided into the following six segments:

Administrative Support

Provides for one Administrative Specialist and one Information Specialist in the Office of the Animal Genetics and Production Branch (AG&PB), Developmental Therapeutics Program, DCT.

Genetic Monitoring

Monitors all of AG&PB contract rat colonies for genetic purity. In addition this contract monitors the starts received from VRB-NIH both pre and post rederivation.

Partial Support for Harlan/Sprague-Dawley

Provides Work Orders and Shared Services type functions for the Harlan Sprague Dawley Animal Production contract. It serves as a method for paying the PRI for services performed at the Animal Production Area.

Tumor Bank

This segment 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*.

In Vitro Cell Line Screening Project

This new project (began in September, 1984) is devoted to the development and operation of *in vitro* disease-oriented antitumor drug screening models based on the use of established human tumor cell lines. This activity is planned to represent the primary DTP drug screening effort and will interface with other components of the drug development program.

Metastatic Models Development

This activity is closely related to the *in vitro* cell line screening project described above. Selected human tumor cell lines are being developed for use in metastatic models for *in vivo* drug evaluations.

RAYLO CHEMICAL, LTD. (NO1-CM3-7635)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

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 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.

RESEARCH TRIANGLE INSTITUTE (NO1-CM3-7619)

Research Triangle Institute is one of the three 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 physicalchemical 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 spectroscopy 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.

RESEARCH TRIANGLE INSTITUTE (NO1-CM3-7636)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SIMONSEN LABORATORIES (NO1-CM3-0230)

This contract furnishes approximately 104,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated January, 1985.

SIMONSEN LABORATORIES (NO1-CM3-7624)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for hybrid production and the many NCI research activities.

SIMONSEN LABORATORIES (NO1-CM5-7729)

This Rodent Production Center contract produces three hybrids (B6C3F1, B6D2F1, CD2F1) under barrier conditions.

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 special investigations. The breeding stock is received from the primary genetic centers. This contract terminated January, 1985.

SISA, INC. (NO1-CM3-7637)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

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 developed hybridomas secreting monoclonal antibodies against human IL-2, and human gamma interferon and has undertaken development of monoclonal antibodies against human and murine tumor necrosis factor, human B-cell growth factor, interleukin-1, and human alpha and beta interferons. This contract will expire September, 1985 and is being recompeted.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CM3-7565)

The purpose of this contract is to provide methods for dose calculations for cancer therapy using radioactively labelled antibodies directed to tumor associated and/or tumor specific antigens. Dose calculations are to be developed for radiation doses to tumors and normal tissues resulting from the use of various isotopes (alpha, beta, or gamma emitters) tagged to antibodies for therapy purposes. Calculational methods are to be correlated with three-dimensional patient anatomy and the distribution and specific activity of radioactivity [as defined by scanners such as CT, single photon emission computed tomography (SPECT), positron emission tomography (PET) or gamma cameras] and should be compatable with conventional radiotherapy treatment planning systems insofar as possible.

SMALL BUSINESS ADMINISTRATION (BIOTECH RESEARCH LABORATORIES) (NO1-CM3-7558)

This contract provides personnel to assist the staff of the Drug Evaluation Branch with the scheduling and evaluation of compounds for antitumor activity in the pre-screen and tumor panel test systems. They also assist in requesting required follow-up testing of compounds of interest and the scheduling of testing for those compounds recommended for further evaluation in additional antitumor test systems. The contractor provides for the coordination and data entry for these compounds to both manual and computerized files which provide management reports for staff to monitor compounds in the linear array.

SMALL BUSINESS ADMINISTRATION (BIOTECH RESEARCH LABORATORIES) (NO1-CM3-7590)

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. Complete records are maintained on all biological materials handled under the contract.

SMALL BUSINESS ADMINISTRATION (BIOTECH RESEARCH LABORATORIES) (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: (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 cells and retroviruses; and (5) testing various tissue cultured cell specimens for mycoplasma contamination.

SMALL BUSINESS ADMINISTRATION (MAXIMA CORPORATION) (NO1-CM4-7628)

The objective of this small business contract is to perform a variety of computer searches such as full structure searches, substructure searches and data item searches in support of the DTP 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 names for compounds of interest.

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.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM3-7576)

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. The contractor 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. Additional effort was added to this contract to provide support for the receipt, organization, and disbursement of all clinical protocols and related materials involved in the Cancer Therapy Evaluation Program protocol review process.

SOUTH FLORIDA, UNIVERSITY OF (NO1-CM3-7639)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SOUTHERN ANIMAL FARMS (NO1-CM3-0227)

This contract furnishes approximately 104,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated January, 1985.

SOUTHERN ANIMAL FARMS (NO1-CM5-7727)

This Rodent Production Center contract produces CD2F1 hybrid mice and the CD8F1 hybrid. In addition, the pure strains BALB/c-CMc and DBA/8 are maintained within this contract.

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. This contract terminated January, 1985.

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

On this contract a Phase I/II clinical trial was conducted, evaluating recombinant beta interferon in the treatment of patients with disseminated malignant disease. One more patient is required for completion. Toxicities have included high fevers up to 40°C lasting for 12 hours at doses ranging from 30-60 million units. Some patients have tolerated the dose escalation schema better than others. This contract expired April, 1985.

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 cancer 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.

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 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. The contractor has completed a task to characterize the preclinical pharmacology of 5-Azacytidine, NSC 102816.

SOUTHERN RESEARCH INSTITUTE (NO1-CM3-7552)

This contract utilizes a variety of murine leukemia and solid tumor models to (1) evaluate the antitumor activity of congeners or prodrugs of new lead compounds; (2) conduct special non-protocol studies on promising compounds in order to identify the best candidates for further development; and (3) evaluate the activity of purified natural products. The special non-protocol studies may include experiments to determine whether the antitumor activity of an antimetabolite can be reversed by endogenous substances and whether congeners retain activity against tumors with acquired resistance to clinical agents. This contract is currently being recompeted.

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 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-CM3-7638)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7580)

This contract provides DCT with a resource to conduct detailed preclinical evaluations of anticancer agents identified as promising on primary screening contracts. Agents are subjected to rigorous testing to select those most deserving of development to clinical trial. In addition, an effort is made to develop treatment strategies to address common causes of treatment failure, such as the growth of tumor cell populations which are resistant to standard agents. As appropriate, studies are conducted in vitro or in vivo against transplantable animal tumors and human tumor xenografts. Principal tasks include the conduct of schedule dependency studies, activity evaluations of clinically formulated products, and evaluations of agents in tests which are different or more challenging than those used in primary screening (e.g., tests in animals bearing advanced tumors).

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7581)

This contract provides assistance to the Drug Evaluation and Animal Genetics and Production Branches in protocol development and quality control of tumor lines and host animals. Tentative protocols are developed for new test systems or refinements suggested to enhance current systems. Animals and tumors used in the screening program are monitored for reproducibility and consistency of response. Cell kinetics are elucidated for in vivo systems utilized by the screening program.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7615)

The objective of the contract is to provide preliminary 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 determine the drug concentration and time of exposure required for significant tumor cell kill. The latter will be used to help treatment schedule selection and dose escalations for Phase I clinical trials.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7646)

This contract provides for the testing of materials in the P388 pre-screen and the mouse host antitumor models in the DCT panel of in vivo test systems at a level of 25,000 L1210 equivalents per year. The contract provides for special studies as requested by the Project Officer such as the detailed evaluation of compounds and the characterization and evaluation of tumor models. All testing is carried out according to the protocols of the Drug Evaluation Branch, DTP. Conventional mouse tumor panel models now in use at this laboratory are the B16 melanoma, the $CD8F_1$ mammary adenocarcinoma, the colon 38, the L1210 leukemia, and the M5076 sarcoma mouse tumor test systems. The panel of test systems is currently being modified, for example, the mouse tumor, B16 melanoma, is being phased out of use and testing with the human tumor, LOX amelanotic melanoma, is being initiated.

SOUTHWEST RESEARCH INSTITUTE (NO1-CM3-7640)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

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 radio-autography 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. The contractor is currently working on a task to characterize the preclinical pharmacology of an L-cysteine Derivative, NSC 303861.

SRI INTERNATIONAL (NO1-CM3-7618)

SRI International is one of the three 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.

SRI INTERNATIONAL (NO1-CM3-7641)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SRI INTERNATIONAL (NO1-CM4-7611)

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). Benzamide 3-hydroxy- (NSC 379289) has confirmed *in vitro* and *in vivo* radiosensitization that is as good if not better than Misonidazole. Benzotriazine dioxides have also demonstrated *in vitro* radiosensitizer activity. These novel leads are being developed.

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 National Center for Devices and Radiological 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-CM3-7642)

This is one of the 12 master contractors 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, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

STARKS ASSOCIATES, INC. (NO1-CM4-7588)

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 C. P. (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 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; and (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.

TACONIC FARMS (NO1-CM3-0226)

This contract furnishes approximately 104,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production center colonies. This contract terminated January, 1985.

TACONIC FARMS (NO1-CM5-7730)

This Rodent Production Center contract will provide athymic nude mice under maximum barrier conditions.

TECHNASSOCIATES, INC. (NO1-CM3-7612)

The purpose of this contract is to establish the need and requirements of the extramural community for an acquisition, quality assurance and distribution program for biological response modifiers. The contractor will conduct a survey to define potential suppliers and general availability of biologics as well as the amount, time intervals, levels of quality assurance, and standardization, toxicity testing, formulations and relevant in vitro and in vivo biological testing required by potential scientific investigators involved in testing and evaluating biologics. The survey is being conducted by phone, mail questionnaires and personal contact. Individuals are being contacted in private industry, academic institutions and government. The final document will be a written report summarizing all survey results and providing conclusions and interpretation. This contract expired June, 1985.

TECHNICAL RESOURCES, INC. (NO1-CM5-7658)

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 logistial support in the development of related or otherwise required documentation and conference support activities of the Division. This contract was awarded in April, 1985 for a five-year period.

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 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.

TEXAS A&M RESEARCH FOUNDATION (NO1-CM3-7536)

This contract montitors 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 National Center for Devices and Radiological 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 is presently conducting Phase I studies with Tiazofurin and Menogaril and has started a new trial with Didemnin. This contract expired July, 1985.

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 AND 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. The Contractor is presently conducting Phase I studies with Trimetrexate and Taxol. This contract expired July, 1985.

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 AND TUMOR INSTITUTE) (NO1-CM7-7149)

This contract provided follow-up support for patients included in three adjuvant clinical trials for non-small cell lung cancer through the Lung Cancer Study Group. Patients were followed at regular intervals as determined by the protocols. This contract expired December, 1984.

THERADEX SYSTEMS, INC. (NO1-CM3-7553)

The object of this contract is to provide a Clinical Trials Monitoring Service for the Phase I/II Cancer Therapy Evaluation Program and Biological Response Modifiers Program investigators and all other investigators using NCI-sponsored investigational drugs. This service has four components: (1) a central data management resource for investigators conducting Phase I clinical trials; (2) a site visit monitoring resource for DCT to assure that Phase I/II contractors are in compliance with federal regulations; (3) co-site visiting cooperative groups as observers of peer audits; and (4) site visit monitoring of all other individual investigators conducting investigational drug trials.

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 National Center for Devices and Radiological 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.

VERMONT, UNIVERSITY OF (NO1-CM2-7547)

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 of regimens mutually agreed upon. The Contractor is presently conducting a Phase I study with Spiromustine and has just started a a new trial with Didemnim. This contract expired July, 1985.

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 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.

VETERANS ADMINISTRATION 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. This Agreement was terminated March, 1985, and the animal program is being transferred to the Frederick Cancer Research Center.

VSE CORPORATION (NO1-CM5-6754)

This contract is in the first year of a five (5) year period. It is the result of a competition held during 1984. Data processing services are provided to the Developmental Therapeutics Program by this contract. The scope of work includes: (1) reducing and disseminating data resulting from the screening program of the Drug Evaluation Branch to staff and suppliers of the compounds being tested; (2) writing new computer programs to process data from new methods of screening; (3) maintaining and modifying existing computer programs so that they are able to run at the Division of Computer Research and Technology; (4) improving data collection methods; (5) documenting all computer programs and Contractor's procedures for data handling and running computer programs; compounds relating to collection and dissemination of data; (7) performing statistical analyses of test systems and test system parameters; (8) extensively collaborating with the Drug Information System Contractor; and (9) participating in scientific meetings.

WARNER-LAMBERT COMPANY (NO1-CM3-7285)

This no-cost contract provides for developing and marketing AZQ as an antitumor agent. Its purpose is to facilitate development of the drug to the NDA stage. Warner-Lambert has conducted both pre-clinical and clinical studies with AZQ. All the additional toxicology studies required for FDA approval and foreign registration have been completed or will be initiated soon. Warner-Lambert defined a comparative trial of AZQ and BCNU in primary main tumors as the most important study to be conducted. The study is now activated and accruing patients.

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 components from the confirmed active beers; and (4) the production of large quantities of antineoplastic agents selected for advanced development. One compound, a pyranone phosphate, is currently in toxicology study. Several other promising candidates are in early development. This contract will terminate in FY 1986.

WARNER-LAMBERT COMPANY (NO1-CM4-7589)

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.

WASHINGTON UNIVERSITY (NO1-CM4-7696)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

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 facility construction was completed in March 1984. The cyclotron was accepted in September 1984 and patient treatment began in November 1984.

WAYNE STATE UNIVERSITY (NO1-CMO-7404)

The Contractor conducts Phase II/III studies in patients with advanced solid tumors. A minimum of 200 patients a year were 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 NCI and the Principal Investigator. Accrual to new protocols ended in January, 1985; treatment and follow-up will continue through January. 1986.

WAYNE STATE UNIVERSITY (NO1-CM2-7551)

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 is presently conducting Phase I studies with Acodazole, Caracemide, and Spiromustine. This contract expired July, 1985.

WISCONSIN, UNIVERSITY OF (NO1-CMO-7434)

This contract is conducting three Phase I/II clinical trials of recombinant interferon in the treatment of patients with disseminated malignancies. Clinical trials with the gamma interferon are now reaching completion. Due to a delay in initiating the trial, this contract has received a no cost extension through June 30, 1985 in order to complete the final third step in the study to evaluate further the biologic effect of gamma interferon.

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. The Contractor is presently conducting Phase I studies with Acivicin, Azodazole, and CBDCA-cisplatinum. This contract expired July, 1985.

WISCONSIN, UNIVERSITY OF (NO1-CM4-7669)

On this contract, a Phase I trial will investigate a single, rapid infusion versus a more prolonged infusion of recombinant interleukin-2 in patients with advanced malignancies. Clinical toxicity, pharmacokinetics and immunologic monitoring will be conducted. This trial begin in early May of this year. This contract will expire October, 1985.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1984 - September 30, 1985

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 Landow Building in Bethesda, 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: Biological Chemistry, Experimental Therapeutics and Metabolism, Pharmacology and Experimental Therapeutics, Tumor Cell Biology and Molecular Pharmacology. 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 1.

During this year a major reorientation of the drug development program has been initiated. A workshop, sponsored by DTP, was held in January entitled "Disease Oriented Antitumor Drug Discovery and Development." This led to a recommendation by a subcommittee of the DCT Board of Scientific Counselors that the essentials of the proposal presented at the workshop be adopted. The full Board approved the recommendation of the subcommittee and a phased implementation of the disease-oriented screen has begun. At the same time a major area of emphasis has been established in the natural products arena. The search for novel compounds has been broadened to encompass

sources such as fungi, bluegreen algae, marine organisms obtained at different ocean depths and plant collections from remote geographical locations such as Southeast Asia. The forthcoming year is expected to see the initiation of several projects in this area and the natural products so obtained will enter the disease-oriented screen of human tumor cell lines.

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 in the DTP.

The DS&CB achieves its central mission by engaging in a variety of Program activities, namely, selective acquisitions, pre-selection model development, task order syntheses, contract syntheses, storage and distribution and computer assisted structure-activity analysis. The DS&CB works closely with the Information Technology Branch in restructuring the automated chemical data base system. In addition, DS&CB supports the Radiation Research Program, the European Organization for Research on Treatment of Cancer Program and the US Japan Cooperative Cancer Research Program. Recently, the DS&CB has assumed the responsibility for the radiolabelled synthesis. A collaborative program has been initiated with the Laboratory of Biological Chemistry for the design and synthesis of inhibitors of phospholipid metabolism.

During the past year as a result of acquisition activities, 360 new suppliers were added to the current list and seven synthetic compounds passed Decision Network (DN) 2A. The congeners and prodrug synthesis contracts have produced two improved drugs, a platinum (IV) complex, NSC 363812, which has advanced to DN 2B and a pyrazinediazohydroxide, NSC 361456, to the DN 2A level.

The new screening approach will undoubtedly have some impact on the activities of DS&CB. In particular, the amounts of material needed for primary screening will be greatly reduced and this may make accessible to the program compounds which are only available in very limited quantities.

b. Natural Products Branch (NPB)

The objectives of the Natural Products Branch are: (a) to acquire a wide spectrum 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 quantities of compounds under study to assure a sufficient supply of material for detailed biological evaluation and subsequent developmental studies including pharmaceutics and toxicology.

During the past year fermentation contractors have evaluated 24,762 new cultures. Of these 5,476 (22%) were active in one or more of the prescreens. About 2,262 (49%) of these active cultures were regrown and retested and 1,619 (60%) were then selected for evaluation in vivo against P388; 346 (21%) were found to be active.

In 1984 372 pure natural products were acquired, of which 344 were donated and the remaining 28 were obtained from contractors. Also, through the literature surveillance program, 165 compounds (44% of new acquisitions) were received in direct response to requests for specific compounds. The overall response rate to the literature requests is 28% and the project continues to be a very cost-effective way of acquiring compounds of interest from around the world.

The commitment of the DTP to the compound-oriented screen will provide the opportunity for the NPB to examine a multiplicity of diverse materials of natural origin for their potential as selective antitumor agents. The future yield of active materials remains a question, but there is no doubt that natural products have an enormous potential for a variety of biological effects and there is every reason to anticipate that some of them will display useful antitumor activity.

c. Drug Evaluation Branch (DEB)

During the past year, 10,554 materials were examined in the in vivo P388 pre-screen. Of these 9,770 were synthetic chemicals and 784 were natural products. Three hundred and forty (3.2%) of the materials screened demonstrated confirmed activity in the pre-screen and entered the secondary phase of evaluation against a spectrum of transplantable mouse tumors.

The human tumor colony forming assay (HTCFA) has continued to be used with emphasis being placed on those compounds which were inactive in the in vivo P388 pre-screen. Small numbers of the materials displayed possible evidence of differential or selective toxicity and will be examined further in other tumor types. It is now very much apparent that the HTCFA is not viable as a primary screen for antitumor activity. Evaluation of its potential as a predicting system for human tumors in vivo will have to await the outcome of clinical trials of those agents identified by the assay. The first of these agents, dihydrolenperone (NSC 343513), will begin Phase I clinical trial in lung cancer patients in the summer.

Work has continued on the development of an in vitro disease-oriented antitumor screening model involving about 30 different human lung cancer cell lines. This Lung Cancer Drug Discovery Project (LCDDP) is the vanguard of the proposed new antitumor screen and it is expected that this LCDDP will become operational in the fall of 1985.

Development of metastatic human tumor models has progressed to the point that metastatic strains of several human tumors from lung, liver and lymph nodes have been selected. The human amelanotic melanoma cell line LOX appears to be particularly well suited for use in experimental metastatic chemotherapy and several lung tumors also appear promising in this regard.

Treatment schedule and/or route dependency studies were completed for five clinical candidate drugs including flavone acetic acid, deoxyspergualin, tetracarcin and an anthrapyrazole derivative. Biochemical studies suggest that deoxyspergualin may require activation by an amine oxidase to an aldehyde before tumor inhibition can occur. Congeners of several drugs are being evaluated for improved properties. Included in these evaluations are congeners of isopropylpyrolizine, camptothecin melamines, triazenes and some cisplatin analogs, in particular, tetraplatin.

There are now four National Cooperative Drug Discovery Groups (NCDDG) which will be in existence in FY 85. The four groups encompass 20 laboratory projects in 14 academic, research, and industrial organizations. The two NCDDGs awarded this fiscal year will investigate topoisomerases as therapeutic targets and the use of antireceptor monoclonal antibodies in cancer treatment. An ongoing collaboration has been established between DEB and the initial two NCDDGs and this relationship satisfactorily fulfills the requirement that the Government actively participate in work conducted under a Cooperative Agreement.

2. Biological evaluation

The primary function of the Animal Genetics and Production Branch (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 the Tumor Repository.

During the past year, in anticipation of the forthcoming changes in the screening program, the AG&PB has begun to make the necessary changes in program emphasis which will be needed to accomodate the new activities. The branch will continue to provide, the quality control measures already in place for all DTP animals, during the transition period.

It has now been established that it is possible to freeze fertilized mouse embryos so that strains not in current use can be alternatively "stored" in this fashion until needed.

3. Formulation and bulk chemical procurement

The Pharmaceutical Resources Branch (PRB) has successfully accomplished its objectives in providing high quality bulk chemicals and pharmaceutical products to the various Programs in the Division of Cancer Treatment. During this reporting period, the prep lab contractors prepared over 181 compounds totaling more than 100 kilograms. Examples of bulk pharmaceutical substances which when delivered for formulationn included: Carboplatin (NSC 241240); 4-Ipomeanol (NSC 349438); Methotrexate (NSC 740); Ara-AC (NSC 281272); Fludarabine (NSC 312887); and L-Buthionine-S, R-sulfoximine (BSO) (NSC 326231). The Clinical Products Section directed the contract production of more than 450,000 injectable units and more than 880,000 oral dosages for clinical distribution. More than 20 BRMs have been received under this project and many more are expected in the next several months. The products received during this period included Interleukin-2; Thymosin; Alpha-Interferon; Lymphoblastoid Interferon; and Monoclonal Antibodies.

The pharmaceutical research and development activities were also quite successful in completing development of nine formulation assignments including parenteral products of Tetraplatin (NSC 349438); anthrapyrazole (NSC-349174); and others.

4. Preclinical toxicology

During this fiscal year the direction of the Toxicology Branch (TB) has shifted from an unidirectional course to two highly integrated paths. The first path continues to be the elucidation of the potential adverse effects of new anticancer agents. The second path is the acquisition and use of pharmacokinetic information to reliably extrapolate toxic effects across species by relating plasma drug levels (peak and steady state) to the appearance and severity of toxicity. Integration of these two courses permits a more rational evaluation of the role of schedule dependence in efficacy of drug as well as in development of toxicity.

Data on the following agents have been, or are anticipated to be, presented to the Decision Network Committee in FY'85.

Flavone Acetic Acid	NSC 347512
Merbarone	NSC 336628
Deoxyspergualin	NSC 356894
Pibenzimol	NSC 322921
Triazine Antifol	NSC 127755

Toxicology studies have been, or are anticipated to be, initiated in FY'85 on the following compounds.

4-Ipomeanol	NSC 349438
Azacytosine Arabinoside	NSC 281272
Anthrapyrazole	NSC 349174
Tetraplatin	NSC 363812
Buthionine Sulfoximine	NSC 326231
Discreet Agent	NSC 339004D
Hexamethylene Bisacetamide (po)	NSC 95580

5. Information technology

A fully operational version of the Drug Information system (DIS) was installed in the Information Technology Branch (ITB) in March 1985. That allowed the NCI contract with the Chemical Abstracts Service (CAS) to be terminated the following month. The NCI-CAS contract had been in existence for a dozen years and its end, with the start-up of the DIS, marks a turning point in computer use in DTP. The DIS represents a consolidation of most of the disparate computer systems used by the DTP; it permits interactive control of acquisition, shipping, inventory and testing of chemicals. The major DIS databases are self-updating and the updating proceeds automatically at pre-determined dates and times. All output from the DIS is directed to a high-speed laser printer in the Landow Building. This printer is capable of graphical and non-graphical printing and represents a major advance over the earlier procedures which required the use of several different printers and which, as a result, could take hours or even days to complete. Since the DIS was released, it has logged tens of thousands of transactions. Numerous minor problems have been encountered and and resolved and many of the important DIS procedures have been greatly optimized. This process has been aided significantly by the installation at DCRT of a third KL-10 processor in the DEC System 10 computer.

In a new excursion, the Branch is involved in the design of a computer system which is to be central to the new cell line project upon which the DTP is embarking. The new data system will run on a series of independent local microprocessors, controlled by and reporting to the DIS. In this project the throughput requirements are very high. Accordingly, a commitment has been made to robotics, which promises to deliver such a high volume of tests.

6. Grants in pharmacology and biochemistry

The Extramural Research and Resources Branch (ERRB) is responsible for the administration of preclinical grant-supported research leading to the development of effective anticancer 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, comparative pharmacology and toxicology, and

mechanisms of drug action. As new findings and significant developments occur, appropriate changes are made in programmatic emphasis and support in each of its major research categories. This Branch maintains liaison and coordinates its research activities with those of other Divisions of the National Cancer Institute.

During FY 85, the Branch supported 283 research projects totalling $38.1 million dollars.

B. Intramural Program

1. Laboratory of Biological Chemistry (LBC)

The Laboratory of Biological Chemistry was established in 1985 to identify as targets for drug design, cellular reactions that are critical to the control of tumor cell proliferation or differentiation. Recent advances in cell biology are evaluated for possible targets. Agents are designed to interact with these targets and are evaluated for biochemical and antitumor effectiveness. An important aspect of this mission is to develop appropriate in vivo systems to evaluate the chemotherapeutic effectiveness of agents shown to be active in simpler in vitro model systems. Accordingly, the Laboratory is involved in identifying endogenous factors present in vivo that modify drug action and influence differential toxicity with the aim of manipulating these factors to enhance antitumor activity. Ongoing research projects were reevaluated and approximately 50% of the resources was applied to non-traditional targets for antitumor drug design and study. These non-traditional targets include early key biochemical events signaling cell proliferation or differentiation. The other 50% of laboratory resources was applied to the study of either traditional targets or active compounds with traditional or unknown mechanisms of action.

Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Projects have therefore been initiated to develop new chemotherapeutic agents to block the action of growth factors. Non-traditional targets selected for drug design and study include: second messengers inositol triphosphate and diacylglycerol; myristoylation of cellular oncogene products; and protein kinase C.

The availability of tissue culture cell lines has made it possible to study the regulation of proliferation and differentiation of specific hematopoietic cell types and the effects on these cells of known or suspected mediators and modulators. It was found previously that retinoic acid (RA) is a potent inducer of terminal differentiation of the human promyelocytic cell line, HL-60, and the human monoblast- and monocyte-like cell lines, U-937 and THP-1. Studies are now in progress to apply the results obtained in vitro with HL-60 to a transplantable HL-60 tumor carried in athymic nude mice.

2. Laboratory of Experimental Therapeutics and Metabolism (LETM)

The LETM encompasses an interdisciplinary research program in tumor biology, pathology, biochemistry, pharmacology and toxicology that include 1) utilization of the biochemical and pathobiological characteristics of hepatic and extrahepatic tumor cell populations to define the histogenesis of specific cancers; 2) establishment of specific animal and human tumor cell lines to study new and existing anticancer drugs for their mechanism of toxicity; 3) investigation of biochemical and molecular mechanisms of drug metabolism and drug toxicity; 4) elucidation of the metabolic and physiological factors that may underlie target organ and target cell specific toxicity and prevention of toxicity by cellular defense mechanisms and 5) the continuation of the development of suitable cellular and animal models to elucidate mechanisms of toxicity of anticancer drugs and chemical modulators of anticancer drug effects.

Although all of the current research is based on information generated in earlier animal experiments, the major emphasis during this report period has been on the characterization and establishment of human lung cancer cell lines (HLCCL) for research on cell type specific chemical/drug interactions. The experience generated through such studies has also enabled the initiation and design of a number of experiments primarily conducted by investigators of the Pharmacology and Toxicology Section, LETM. In summary, it was found that:

1. Of seven investigated non-small cell derived (HLCCL), only two adenocarcinomas (NCI-H322 and NCI-H358) exhibited unequivocal well differentiated features of one pulmonary cell type (as assessed by electron microscopy) while all others demonstrated either dual differentiation (e.g., adenosquamous) or poor differentiation which made a diagnosis by cell type questionable. Evidence was also found that well differentiated lung tumors undergo pronounced morphological changes during prolonged maintenance *in vitro*. This exemplifies the need for careful electron microscopic assessment of HLCCL before and during their use for biochemical and pharmacological experiments.

2. Similar to *in vivo* studies in hamsters, the pulmonary carcinogen, diethylnitrosamine (DEN), is preferentially metabolized by HLCCL with features of pulmonary Clara cells *in vitro* and such metabolism is inhibitable by inhibitors of cytochrome P-450 enzymes.

3. HLCCL of different cell types metabolize DEN via different enzyme systems, e.g., Clara cells via cytochrome P-450 and alveolar type II cells via prostaglandin endoperoxide synthetase. Cell type specific differences such as this in nitrosamine activation may well be responsible for the known organ and cell specific carcinogenicity of these compounds.

4. Similar to experiments in rats, the pulmonary toxin and candidate anticancer drug, 4-ipomeanol, is preferentially metabolized by an HLCCL with characteristics of Clara cells and such metabolism is mediated by cytochrome P-450 enzyme systems. The selective toxicity of the compound for this cell line suggests that its usefulness as an anticancer drug for pulmonary adenocarcinomas and other solid tumors of similar cell types should be pursued.

5. The xenobiotic-metabolism enzymes, cytochrome P-450, arylhydrocarbon hydroxylase and ethoxycoumarin O-deethylase activity are selectively present in non-small cell derived HLCCL.

6. Normal alveolar type II cells of rat lung proliferate in vitro for 7 days in culture, retain their typical ultrastructure and phospholipid synthesis and can be identified by immunoperoxidase using an antiserum specifically for rat surfactant apoprotein.

3. Laboratory of Pharmacology and Experimental Therapeutics (LPET)

LPET (formerly 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. In addition, the laboratory as a whole develops analytical methodology for the detection of antitumor drugs and their metabolites, and also participates actively in the clinical pharmacology/Phase I trials of new agents which either originated within the laboratory or which were derived from extramural sources but in whose preclinical development the laboratory has played a major role. Included among the many investigations which have been conducted during the reporting period are the following studies: Arabinosyl-5-azacytosine (ara-5-AC), a compound originally synthesized within LPET and presently under active development toward clinical trial, exhibits unexpectedly high intracerebral antitumor activity in the IC L1210 murine test system (i.e., activity greater than that of ara-C, and comparable to that of the prototype reference compound, BCNU). A rapid and sensitive HPLC assay for the quantitation of ara-5-AC in biological fluids has been developed. These findings will be of direct applicability in the projected Phase I/II clinical trials of this compound shortly to be initiated by DCT.

All the possible phosphonate analogs of the active metabolite of TAD (the active anabolite of tiazofurin) have now been prepared. One of these (beta-methylene TAD) was equipotent to TAD as an IMPD inhibitor and possesses the advantage of being resistant toward cleavage by TAD-inactivating enzymes.

In studies of the differences between metastatic cells and their non-metastatic counterparts, an L1210 system utilizing tumor cells isolated from murine liver has been utilized. Marked differences have been observed between liver metastatic L1210 tumor cells and their ascitic counterparts, e.g., a 2-fold increase in glutathione concentration and a 5-fold greater level of surface SH-groups in the liver metastatic cells. The metastatic cells are more resistant to melphalan, but can be resensitized by blocking the surface sulfhydryls with 6,6-dithionicotinic acid.

The new agent, merbarone (NSC 336629), has been extensively examined in terms of possible mechanism of action. The compound is an effective but atypical inhibitor of DNA synthesis; among a number of factors examined, this inhibition appears to correlate most closely with the ability of the compound to generate single-strand DNA breaks which do not, however, appear to be protein-associated. Investigation of the molecular pharmacology of this unusual wide-spectrum antitumor agent is continuing.

4. Laboratory of Tumor Cell Biology (LTCB)

While the overall 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, the major activities during this reporting period have focused on studies relating to the proposed etiologic agent of AIDS namely, HTLV-III. Among some of the many accomplishments by the laboratory are:

1. More than 100 isolates of HTLV-III have been obtained from patients with acquired immune deficiency syndrome (AIDS) and pre-AIDS.

2. Seroepidemiological studies with HTLV-III show that 95% of the AIDS and pre-AIDS patients and approximately 45% of healthy homosexuals carry HTLV-III antibodies.

3. Development of an assay for detection of neutralizing antibodies to HTLV-III in sera of individuals exposed to the virus.

4. Studies on the development of an animal model indicate chimpanzees to be a suitable animal. Seroconversion and HTLV-III isolation was demonstrated in chimps infected with HTLV-III.

5. Several drugs, including foscarnet, cis-platinum and lapachone derivatives have been shown to inhibit HTLV-III replication.

6. Determined the nucleotide sequence of the envelope gene of HTLV-III (RF).

5. Laboratory of Molecular Pharmacolgoy (LMP)

A major goal of the LMP is to develop strategies for the selective killing of particular human tumor cell types. Studies are being conducted in three major areas namely, 1) the mechanisms of action of alkylating agents; 2) topoisomerase II as a target for DNA intercalating agents and epipodophyllotoxins; 3) regulation of chromosomal protein biosynthesis in relation to the control of cell proliferation.

The alkylating agent studies have shown the following: 1) there are marked differences in reactivity among guanines in a DNA sequence; 2) the differences are not markedly dependent on the solvent conditions of reactions; 3) the guanine selectivity pattern is different for different nitrogen mustards; 4) the potential inter-strand crosslinking sites -- e.g. 5'-GC-3' -- show relatively low reactivity for HN2, melphalan and spiro-hydantoin mustard; 5) these sites, however, show a markedly enhanced reaction with uracil mustard; 6) runs of G's (potential sites of intra-strand crosslinking) exhibited disproportionately high reactivity with most nitrogen mustards; 7) quinacrine mustard exhibited a uniquely different pattern of reactions.

Topoisomerase II studies have shown that the enzyme's activity measured by the protein-associated strand break response is dependent on the proliferation state of the cells. In non-proliferating 3T3 cells, this activity was much less than in proliferating cells. When quiescent cells were stimulated to proliferate, the topoisomerase II response increased at about the time of the first wave of DNA synthesis, but did not then oscillate in phase with the cell cycle. Thus the observed alteration in topoisomerase II activity appears to be related to the proliferation state of the cells and not to phase of the cell cycle.

Investigations of chromosomal protein synthesis and cell proliferation have resulted in further insight on the mechanism of the regulation of DNA and histone synthesis during S-phase. Studies were carried out of the effects of protein synthesis inhibitors applied to cells at relatively low doses such that protein synthesis was reduced by only 50%. Although DNA and histone syntheses were initially inhibited proportionately, these syntheses then recovered despite the continued inhibition of overall protein synthesis. This was found to be brought about by a compensatory elevation of histone m-RNA. A mechanism thus exists which tends to preserve the duration of S-phase when G1 is lengthened because of nutritional or metabolic deficiencies. The mechanism will be studied further by using other means to lengthen G1 phase. The relevance to cancer chemotherapy is that this metabolic regulatory mechanism may be defective in some neoplastic cell lines and could lead to a strategem by which such cells could be selectively killed.

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/84 - 3/31/85)

NSC Number	Name	Compound Type*
	Decision Network 2A	
201047D	Discreet	S
320486D	Discreet	S
321803	Estra-1,3,5(10)-trien-17-one, 3-methoxy-4-nitro-; Nitroestrone	S
332598D	Discreet	NP
338720D	Discreet	S
338947	Methanesulfonic acid, (methylsulfonyl)-, 2-chloroethyl ester	S
344007	Piperazine, 1-(2-chloroethyl)-4-(3-chloropropyl)-, dihydrochloride	S
348948	1,5,2,4-Dioxadithiepane, 2,2,4,4-tetraoxide	S
349156	Pancratistatin	NP
349174	Anthra[1,9-cd]pyrazol-6(2H)-one, 5-[(3-aminopropyl) amino]-7,10-dihydroxy-2-[2-[(2-hydroxyethyl)amino] ethyl]-, hydrochloride (10:17)	S
355644	Anthra[1,9-cd]pyrazol-6(2H)-one, 7,10-dihydroxy-2-[2-[(2-hydroxyethyl)amino]ethyl]-5-[[2-(methylamino) ethyl]amino]-,acetate (salt) hydrobromide (10:5:21)	S
363812D	Discreet	S
366241	7H-Pyrido[4,3-c]carbazolium, 2,2'-([4,4'-bipiperidine]-1,1'-diyldi-2,1-ethanediyl)bis[1-methoxy-, dimethanesulfonate, disulfate; Pyridocarbazolium derivative	S
368390D	Discreet	S

NSC Number	Name	Compound Type*
	Decision Network 2B	
127755	Ethanesulfonic acid, cpd. with 3-chloro-4-[4-[2-chloro-4-(4,6-diamino-2,2-dimethyl-s-triazin-1(2H)-yl) phenyl]-butyl]benzenesulfonylfluoride (1:1); Dihydrotriazine derivative	S
281272	1,3,5-Triazin-2(1H)-one, 4-amino-1-B-D-arabinofuranosyl-; Ara AC (5-Azacytosine Arabinoside)	S
303812	Glycine,[tetradecahdyro-3,9-dihydroxy-4 (hydroxymethyl)-4,11b-dimethyl-8,11a-methano-11aH-cycloheptal[a]naphthalen-9-yl]methyl ester, hydrochloride, (3A,4A,4AA,6AB,8B,9B,11AB,11BB)-; Aphidicolin Glycinate	SS
303861	ethyl ester, methylcarbamate (ester), monohydrochloride; L-Cysteine analog	NP
308847	1H-Benz[de]isoquinoline-1,3(2H)-dione, 5-amino-2-[2-(dimethylamino)ethyl]-; Benzisoquinolinedione	S
336628D	Discreet	S
339638	2H-Pyran-2-one, 5,6-dihydro-6-[3,6,13-trihydroxy-3-methyl-4(phosphonooxy)-1,7,9,11-tridecatetraenyl]-monodsodium salt; Pyranone phosphate	NP
347512	4H-1-Benzopyran-8-acetic acid, 4-oxo-2-phenyl-; Flavone Acetic Acid	S
356894	Heptanamide, 7-[(aminoiminomethyl)amino]-N-[2-[[4-[(3-aminopropyl)amino]butyl]amino]-1-hydroxy-2-oxoethyl]-, (+)-; Deoxyspergualin	NP

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/84 - 3/31/85)

NSC Number	Name	Compound Type*
	Decision Network 3	
322921	Phenol, 4-[5-(4-methyl-1-piperazinly)][2,5'-bi-1H-benzimidazol]-2'yl, trihydrochloride; Pibenzimol	S
325319	Didemnin B	NP
352122	D-Glucuronic acid, cpd. with 5-methyl-6-[[(3,4,5-trimethoxyphenyl)amino]methyl]-2,4-quinazolinediamine (1;1); Trimetrexate	S
	Decision Network 4	
3051	N-Methylformamide	S
264880	1,3,5-Triazin-2(1H)-one, 4-amino-5,6-dihydro-1-B-D-ribofuranosyl-, monohydrochloride; Dihydro-5-azacytidine	SS
267469	5,12-Naphthacenedione, 10-[(4-aminotetrahydro-2H-pyran-2-yl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-, hydrochloride, (8S-cis)-; 4-Deoxydoxorubicin	SS
312887	9H-Purin-6-amino, 2-fluoro-9-(5-O-phosphono-B-D-arabinofuranosyl)-; Fludarabine Phosphate (2-F-ARA AMP)	S
	Decision Network Special	
324360	Benzamide, N,3,4-trihydroxy-; Hydroxamic Acid derivative	S

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/84 - 3/31/85).

NSC Number	Name	Compound Type*
	Decision Network PROD	
45410	Pyrazole	S
57155	N,N"-bis[p-(N'-methylamidino)phenyl]-terephthalamidine; Tetrahydrochloride	S
177248	1,2,4-Triazin-3(4H)-one, 4-B-D-ribofuranosyl-, 1-oxide; Uricytin	NP
364989	1-Bis-(B-chloroethyl)amino-2-aminoethane	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, 1984 - September 30, 1985

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. The DS&CB works closely with the Information Technology Branch (ITB) in restructuring the automated chemical data base system. In addition, DS&CB supports the Radiation Research Program (RRP), the European Organization for Research on Treatment of Cancer (EORTC) Program and the U. S. Japan Cooperative Cancer Research Program. Recently, the DS&CB has assumed the responsibility for the radiolabelled synthesis. A collaborative program has been initiated with the Laboratory of Biological Chemistry for the design and synthesis of inhibitors of phospholipid metabolism.

The DS&CB is gearing up in a variety of ways for the new opportunities for drug discovery through the emerging in vitro based, disease - oriented screens.

The contracts managed by DS&CB are outlined in Table I. Presently, the DS&CB is staffed with six professionals and three 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) 360 new suppliers were added and (b) seven synthetic compounds passed DN2A; NSC's 95678, 291643, 326231, 343513, D-339004, D-339736 and D-361456.

The Acquisition Section worked closely with ITB in restructuring the automated chemical data base system. The acquisition contractor performed

the difficult task of analyzing and reporting system problems for correction as each new computer subsystem was being introduced.

The acquisition team has organized an intense effort to inform the scientific community of the new disease-oriented drug discovery approach. Compounds with biological rationale are being identified for evaluation in the new screens.

Preselection Model

Unless specially targeted, all potential acquisitions are rated for novelty and antitumor activity by computer programs based on molecular structure fragments. The programs are generated from compounds tested in P388, L1210, and B16. The novelty and activity ratings have been especially useful as P388 testing has been reduced. Refinements, modifications and continued development of the programs are conducted as an intramural research project.

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 become feasible because of the chemistry-biology interlink. Examples of structure-activity analyses include: barbiturates, purines and pyrimidines.

Resynthesis

The Task Order mechanism is a cost-effective means of resynthesizing compounds of program interest that are not available in sufficient quantity from the original suppliers. Currently, we have 12 master contractors who have the expertise to synthesize a wide variety of organic and inorganic compounds. For this reporting period, we have awarded 20 individual projects consisting of 135 compounds. Compounds selected for synthesis include PS actives, toxics, LE actives, radiosensitizers/radioprotectors, special requests from intramural scientists, panel compounds, rationally designed compounds and bio-active compounds emerging from literature surveillance. During this reporting period, approximately 125 compounds synthesized through this mechanism were received.

Synthesis of Congeners and Prodrugs

The objective of this project is to advance promising leads that are flawed by limited solubility, stability or narrow spectrum of activity to DN2 level. A variety of approaches are brought into play to overcome these shortcomings: qualitative and in selected cases, quantitative structure-activity estimations, Craig plots, design of isosteres, prodrugs, and congeners. Examples of compounds under development are given below. NSC 363812, a platinum (IV) complex has advanced to DN2B and the pyrazinediazohydroxide, NSC 361456, to DN2A level. A third compound, NSC 373965, a water-soluble phenylalanyl derivative of mitindomide, is expected to advance to DN2 A/B level shortly.

A collaborative program has been initiated with the Laboratory of Biological Chemistry for the design and synthesis of fradulent cyclitols as inhibitors of phospholipid metabolism.

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, approximately 2,000 full structure and 400 substructure searches were performed. In addition, the Questel/Darc and Dialog data base systems were utilized to access synthesis methods for selected compounds. Systematic nomenclature was generated for 700 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 tasks order synthesis.

The contract with the Japanese Foundation for Cancer Research assists DS&CB in identifying potential compounds for acquisition from Japan.

Storage and Distribution

This project provides the service needed by the Developmental Therapeutics Program for the storage, inventory, distribution and documentation of synthetic substances, crystalline natural products and bulk chemical drugs. Flow laboratories, Inc. has shipped more than 15,000 compounds to contract screeners, formulation laboratories, NCI and NIH researchers and investigators in 45 states of the U. S. and 25 foreign countries. Flow has also processed (weighed, recorded and shelved) approximately 14,000 compounds that were returned by the screening laboratories. The Contractor interacts closely with members of DS&CB, DEB, ITB and PRB as well as the acquisitions contractor and DIS contractor. The Contractor is being equipped for weighing a large number of small samples for the forthcoming in-vitro screens.

Radiation Research Program Support

DS&CB works in close cooperation with the RRP and the Radiosensitizer/Radioprotector (RS/RP) Working Group. The Branch monitors the radiosensitizer synthesis contract; and also identifies and acquires novel compounds for the RS/RP screening contract. Three non-nitro compounds, a quinoxaline oxide and two benzamides have demonstrated radiosensitization as effective as misonidazole both in vivo and in vitro and are undergoing detailed evaluation. Phase I trials on NSC-301467 (SRI2508) are nearly completed and protocols are being developed for Phase II trials. Both the synthesis and screening contracts are being recompeted.

TABLE I

CONTRACTS - FY 85

Contractor	Investigator	Contract No.
Alabama, University of	Baker	NO1-CM-27571
Dynamac Corporation	Gray	NO1-CM-37563
Flow Laboratories, Inc.	Dorian	NO1-CM-27505
Georgia Institute of Technology	Zalkow	NO1-CM-27517
Japanese Foundation for Cancer Research	Sakurai	NO1-CM-36011
Maxima Corporation	Sobers	NO1-CM-47628
Research Foundation of The State Univ. of N. Y. at Buffalo	Anderson	NO1-CM-27570
Research Triangle Institute	Kepler	NO1-CM-27515
SRI International	Lee	NO1-CM-47611
Starks C.P., Inc.	Schultz	NO1-CM-47608

12 Master Agreements (Task Order) Contracts:

Alabama, University of	Baker	NO1-CM-37631
Eagle-Picher Industries, Inc.	Roth	NO1-CM-37634
Franklin Research Institute	Kauffman	NO1-CM-37632
Michigan Technological Univ.	Swartz	NO1-CM-37633
Raylo Chemicals, Ltd.	Muhs	NO1-CM-37635
Research Triangle Institute	Seltzman	NO1-CM-37636
SISA, Inc.	Razdan	NO1-CM-37637
Southern Research Institute	Montgomery	NO1-CM-37638
South Florida, Univ. of	Owen	NO1-CM-37639
Southwest Research Institute	Lyle	NO1-CM-37640
SRI International	Smith	NO1-CM-37641
Starks Associates	Starks	NO1-CM-37642

PUBLICATIONS BY STAFF:

1. Paull, K. D., Nasr, M., and Narayanan, V. L. Computer Assisted Structure-Activity Correlations I: Evaluation of Benzo (de) Isoquinoline-1,3-diones and Related Compounds as Antitumor Agents. Arzneim.-Forsch./Drug Res., 34 Nr.10, 1243-1246, 1984.

2. Nasr, M., Paull, K. D., and Narayanan, V. L. Computer Assisted Structure-Activity Correlations II: - Unsaturated Ketones, Lactones, Lactams and Related Michael Type Acceptors as Antitumor Agents. In Garattini, S., Goldin, A., Hawking, F., Schnitzer, R. J. (Eds.): Adv. Pharmacol. Chemother. New York, Academic Press, Vol. 20, 1984, pp. 123-189.

3. Johnston, T. P., Kussner, C. L., Carter, R. L., Frye, J. L., Lomax, N. R., Plowman, J., and Narayanan, V. L.: Studies on Synthesis and Anticancer Activity of Selected N-(2-Fluoroethyl)-N-nitrosoureas. J. Med. Chem.: 27 1422-1426, 1984.

4. Nasr, M., Paull, K. D., and Narayanan, V. L.: Computer Assisted Structure-Anticancer Activity. Correlations of Carbamates and Thiocarbamates. J. Pharm. Sci. In Press

5. Paull, K. D., Hodes, L., and Simon, R. M. Efficiency of Large-Scale Antitumor Screening Relative to Activity Criteria. J. Natl. Cancer Inst., In Press

6. Brewer, A. D., Minatelli, J. A., Plowman, J., Paull, K. D., and Narayanan, V. L. 5-(N-Phenylcarboxamide)-2-Thiobarbituric and (NSC 336628). Biochem. Pharmacol. In Press

PUBLICATIONS BY CONTRACTORS:

During this year, contractors and subcontractors wrote four articles which involved NCI support.

SEMINARS:

1. "Synthesis of Mitomycin C Analogs". Professor D. N. Reinhoudt, Twente University of Technology, The Netherlands.

2. "Second Generation Anthracyclines". Professor Federico Arcamone, Farmitalia Carlo Erba, Milan, Italy.

3. "Pyrimidines With New Biological Roles". Dr. Malcolm Stevens, Professor of Experimental Chemotherapy, Pharmacy Dept., University of Aston in Birmingham, England.

4. "Chemistry of 1,4,2-Dithiazines, Isomeric 1,3-Dithiolimines and Related Heterocycles With 1,2-Dithioethene Structure". Professor Egon Fanghanel, Technische Hochschule, Carl Schorlemmer, Leuna Merseburg, East Germany.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07101-10 DSCB

PERIOD COVERED: October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (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, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

Molecular structure fragments and biological test results from those systems with sufficient data are fed to computer programs that provide estimates of antitumor activity and novelty. These estimates have been used to aid selection since 1980 and are especially useful as the number of compounds screened has been reduced repeatedly during the course of this project.

The selection programs have been integrated into the pre-registry module of the Drug Information System.

The programs have also provided a pioneering effort in automated literature surveillance by running all CAS registrations. This has been performed for all compounds registered in 1977, the year before DTP began its own literature searches.

Also an innovative approach is being investigated combining physical parameters with structure features for our large diverse set of compounds. Early results with the octanol water partition coefficient are encouraging.

PHS 6040 (Rev. 1/84) GPO 904-917

ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 - September 30, 1985

One of the major objectives of the Division of Cancer Treament 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) acquisition of crude biological materials of plant, marine, and microbial origin for the DTP screening program; (2) contract research directed toward isolation of new agents from active extracts; (3) world-wide literature surveillance, and acquisition of natural products with demonstrated biological activity or novel structural types for evaluation; (4) procurement and preparation of large quantities of active agents for drug formulation, tumor panel testing, toxicology and clinical studies.

This year, with the commitment of the Developmental Therapeutics Program to a new direction in in vitro screening, a renaissance of interest in natural products as potentially highly selective antitumor agents has begun with the approval by the Board of Scientific Counselors of a major new program effort in collection of natural products from a wide variety of sources, including terrestrial plants, marine invertebrates, algae and marine microorganisms. The Branch is currently very actively engaged in preparing the Requests for Proposals which will be issued for this work in FY '86.

Objectives

The objectives of the Natural Products Branch are: (a) to acquire a wide spectrum 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 quantities of compounds under study to assure a sufficient supply of material for detailed biological evaluation and subsequent developmental studies including pharmaceutics and toxicology.

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 requires 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	Claridge	NO1-CM3-7556	Fermentation
Microbial Chemistry Research Foundation	Umezawa	NO1-CM4-7593	Fermentation
Polysciences, Inc.	Boettner	NO1-CM3-7557	Plant Products
Warner-Lambert	French	NO1-CM3-7614	Fermentation
Univ. of Illinois	Farnsworth	NO1-CM3-7553	Literature Surv.
(In Competition)		NO1-CM5-7692	Fungal Fermentation

Fermentation Section

The objective is the discovery, isolation and development of novel antineoplastic drugs derived from microbial fermentations. The section maintains four contracts in support of these tasks. Three contracts are devoted to the isolation of unique organisms and systematic evaluation of 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 an attempt to generate numerous new structural leads. *In vitro* prescreens are also constantly improved or changed with emphasis towards the use of mammalian cell lines. The objective is to increase the efficiency of the program in selecting potential leads and to 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 murine and human cell lines (e.g. human bronchioloalveolar carcinoma, L1210 murine leukemia and retrovirus transformed rat kidney cells).

Active fermentations in the prescreens are tested in P388 *in vivo* and confirmed active leads are assigned to chemists 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 three contractors. This year the prescreens allowed the fermentation contractors to evaluate 24,762 new cultures. Over 22% (5476) of these cultures were active in one or more of the prescreens. About 49% (2262) of these prescreen actives were regrown and retested for secondary screening, of which over 60% (1619) were selected for evaluation in vivo against P388, of which 21% (346) are active.

The PRI-FCRF contract has been modified as a shared services contract and is being utilized primarily for pilot plant research and development, and for production of active agents in quantities sufficient for toxicology and clinical trials. About 1.77 million international units of Erwinia asparaginase have been produced, which is equivalent or better in purity than that currently used in the clinic. Six grams of 99% virenomycin have been produced to complete tumor panel evaluation. Attempts are continuing to produce prodrugs of Fredericamycin. A new contract is being awarded in the area of fungal fermentation, which will emphasize the evaluation of fermentations from unusual organisms. New projects have been approved in the areas of isolation and fermentation of marine microorganisms and blue-green algae.

Plant and Animal Products Section

The major function of this program is the acquisition of new active antineoplastic agents from higher plants and animal products (mainly marine). This has largely been achieved by provision of in vivo screening services to NIH grantees and interested scientists worldwide, whereby high priority leads have been established, while subsequent fractionation and isolation procedures have been aided by in vitro bioassay services. Valuable guidance in the collection of new plants and marine organisms has been provided by reference to data in the extensive NCI files on natural products already tested. The scope of the acquisition program will be greatly expanded by the implementation of new plant and marine animal collection programs, and specifications for requests for proposals (RFPs) from prospective contractors in these areas have been drawn up. Organisms collected in these programs will be extracted by a facility under contract to NCI, and extracts tested in the new in vitro primary screens based on panels of human tumor cell lines. The increased sensitivity of the new screens will have a dramatic impact on the role played by natural products in the drug discovery program in that the number of novel active naturally derived agents will be greatly increased. It is planned to establish an in-house isolation group to concentrate on the isolation and purification of compounds exhibiting selective activity, while other active leads will be investigated by external research groups in collaboration with NCI. As before, pure compounds will be evaluated in NCI screens, and any additional quantities required for further investigation will be procured by purchase from the investigator, or will be produced by the contract which is maintained for scale-up isolations. This contract continues to provide valuable support in the production of large quantities of plant-derived drugs required for preclinical and clinical investigations, including taxol, pancratistatin and camptothecin.

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 institutes 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 1984 a total of 372 new pure natural products were acquired, of which 344 (92%) were donated and the remaining 28 compounds (8%) were from contractors.

The literature surveillance program continues to be successful in bringing in new materials for screening. In 1984 165 compounds (44% of new acquisitions) were received in direct response to requests for those specific compounds, while a substantial number of unsolicited compounds received have come from suppliers who were contacted by our program previously in regard to other compounds. The response rate to our literature requests is 28% with 18% of responders supplying the requested compounds and another 10% sending regret letters or supplying other compounds or making inquiries about the program.

Additional details are found in Table 2 which shows that foreign suppliers are currently the source of 56% of the input. The number of compounds acquired for screening is up 22% over last year.

Table 2.

Pure Compounds Acquired in Calendar Year 1984

Compounds by Type and Source

	Industry	Univ.	Res. Inst.	Other	Domestic	Foreign
Plant	9 (4%)	167 (74%)	39 (17%)	9 (4%)	88 (39%)	136 (61%)
Fermentation	55 (58%)	26 (27%)	13 (14%)	1 (1%)	41 (43%)	54 (57%)
Animal	0	53 (100%)	0	0	46 (87%)	7 (13%)

Compounds by Type and Mechanism

	Grant	Contract	Lit. Sur.	Unsol.
Plant	48 (21%)	1 (<1%)	113 (50%)	62 (28%)
Fermentation	2 (2%)	27 (28%)	46 (48%)	20 (21%)
Animal	43 (81%)	0	5 (9%)	5 (9%)

Accomplishments

A major new program in collection of natural products for screening, extraction and storage of extracts, and isolation of active constituents from these extracts has been developed and approved as a major new FY '86 initiative of the Division of Cancer Treatment. The collection projects include plant collection contracts in Africa, Asia and South America, marine organism collections in the Indo-Pacific region, a deepwater marine collection project and fermentation projects utilizing blue-green algae and marine microorganisms. The first of these contracts, in the area of fungal fermentation, will be awarded at the end of FY '85 and is expected to be a valuable source of new bioactive compounds.

Natural Products have continued to be an important source of compounds in preclinical development and early clinical studies as outlined in Table 3.

Collaborative programs have been initiated with outside investigators to develop improved second generation drugs which are designed to obviate the problems with the parent molecules. One project is underway with Dr. Leon Zalkow of Georgia Technical University and Dr. Garth Powis of the Mayo Clinic on analogs of indicine N-oxide designed to increase antitumor activity and decrease hepatotoxicity. Several compounds have been produced which are both more active and more potent than the parent and these are now undergoing preliminary hepatotoxicity evaluation. A second project is being done in the area of camptothecin derivatives with Dr. Monroe Wall of the Research Triangle Institute which has resulted in discovery of several new compounds with increased activity over the parent compound.

A program to develop more soluble analogs of taxol is a high priority effort and two approaches have been taken, one through a collaborative arrangement with Dr. David Kingston at Virginia Polytechnic Institute and the second jointly with the analog and congener development program of the Drug Synthesis and Chemistry Branch. Several compounds from the effort are showing preliminary promise.

A total of 19 compounds were added to the tumor panel as a result of activity in the P388 prescreen or as bypass compounds based on other biological activities. Data on several of these are encouraging and we anticipate having two or three new Decision Network 2A candidates in the next year for advanced preclinical development.

Didemnin B, the first antitumor agent of marine origin, entered clinical trials this year as has taxol a potent plant derived antimitotic agent. An INDA filing is expected early next year for deoxyspergualin, an interesting polyamine analog.

Table 3.

Natural Products in Advanced Development

NSC#	Drug	Origin	Status
163501	Acivicin	Fermentation	Phase II Clinical Trial
218321	Pentostatin	Fermentation	Phase II Clinical Trial
526417	Echinomycin	Fermentation	Phase II Clinical Trial
141633	Homoharringtonine	Plant	Phase II Clinical Trial
125973	Taxol	Plant	Phase II Clinical Trial
269148	Menogaril	Fermentation	Phase II Clinical Trial
325319	Didemnin B	Marine Animal	Phase I Clinical Trial
328426	Phyllanthoside	Plant	Pharmacology
356894	Deoxyspergualin	Fermentation	Toxicology
303812	Aphidicolin Glycinate	Fermentation	Pharmacology
339638	CI-920	Fermentation	Toxicology
349156	Pancratistatin	Plant	Formulation
332598	Rhizoxin	Fermentation	Formulation
364372	Elactocin*	Fermentation	Formulation

* Passed Decision Network 2A FY '85

Staff Publications

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2. Suffness, M. and Cordell, G.: Antitumor alkaloids. In Brossi, A. (Ed.): The Alkaloids, New York, Academic Press, 1985, in press.

3. Marsh, J.C., Shoemaker, R.H., and Suffness, M.: Stability of in vivo P388 leukemia model in evaluation of antitumor activity of natural products. Cancer Treat. Rep. 69: 1985, in press.

4. Foster, B.J., Clagett-Carr, K., Shoemaker, D.D., Suffness, M., Plowman, J., Trissel, L.A., Grieshaber, C.K., and Leyland-Jones, B.: Echinomycin: The first bifunctional intercalating agent in clinical trials. Invest. New Drugs, in press.

ANNUAL REPORT OF THE DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

Drug Evaluation Branch (DEB) objectives are discovery of new anticancer agents, conduct of preclinical 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 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. Program was implemented through 16 contracts funded at more than $6,000,000 (Table 1). This report includes findings through March 31, 1985. The contractors contributing to specific findings are cited in the text by numbers in parentheses as listed in Table 1.

Yield of New Agents for Development to Clinical Trial

Eight agents were referred to the DCT Decision Network (DN) Committee as candidates for development to clinical trial (Table 2). Reports on the preclinical therapeutic activities of Didemnin B (NSC-325319), Nafidimide (NSC-308847), and the differentiating agent, HMBA (NSC-95580) were submitted to the Cancer Therapy Evaluation Program for inclusion in INDA's and Clinical Brochures. Preclinical findings for Dihydrolenperone (NSC-343513) were submitted to CTEP and to the NCI-Navy Medical Oncology Branch to aid in the design of Phase I clinical trials.

In Vivo Screening

In vivo screening (1-5) was initiated for 10,554 materials (9,770 synthetic and 784 natural products). Transplantable mouse leukemia P388 remained the initial in vivo screen (pre-screen). Three hundred forty materials demonstrated confirmed activity in the pre-screen. Agents active in the pre-screen and materials selected to "bypass" the pre-screen because of special interest were tested against a spectrum of transplantable mouse tumors. This spectrum of secondary in vivo models is being modified concomitant with a progressive decrease in the level of in vivo screening and development of a project for screening large scale in vitro against human tumor cell lines at the Frederick Cancer Research Facility (FCRF). At the beginning of this reporting period, the secondary in vivo mouse tumor models included B16 melanoma, L1210 leukemia, M5076 sarcoma, and the MX-1 human mammary carcinoma xenograft. Other murine mammary and colon tumors have been used selectively. The human amelanotic melanoma xenograft, LOX, is being phased into use in lieu of B16. Additional human tumor xenografts are being derived from the human tumor cell lines under development (9, 11). These will be used selectively for in vivo screening of in vitro active materials. Studies related to tumor quality control and establishment of in vivo testing protocols were initiated for human large cell lung carcinoma H-460, lung adenocarcinoma A-549, glioma U-251 and medulloblastoma TE-671, as well as LOX

(9, 11). An in vivo model of human promyelocytic leukemia HL-60 was developed to complement in vitro studies of differentiation inducers (2). A variant of mouse leukemia P388, developed resistant to Adriamycin, has demonstrated pleiotropic resistance to other large molecular weight agents and contains an abnormal membrane glycoprotein associated with such resistance (13).

In Vitro Screening Using Human Tumor Colony Forming Assays

Emphasis was placed on HTCFA testing of P388 in vivo inactive compounds (6-8). Sixty were found active in initial single concentration HTCFA tests and 32 retained in vitro response rates of >20% in subsequent dose-response tests. Of the latter, 23 were tested in a P388 colony forming assay. Eight were inactive suggesting the possibility of differential cytotoxicity to human tumor cells in primary culture. Two compounds demonstrated non-uniform (selective) activity among the six human tumor types used in the HTCFA. Dihydrolenperone (NSC-343513) was selectively active against lung tumors and will begin Phase I clinical trial by the NCI-Navy Medical Oncology Branch. NSC-340307 showed some selectivity against human breast cancer. Selected compounds, chosen for development on the basis of in vivo mouse tumor screens, were tested in the HTCFA to aid in identifying those with potential human tumor type selectivity.

Development of an In Vitro Disease-Oriented Antitumor Drug Screening Model

Work was initiated in October, 1984 at the FCRF (9) to develop in vitro disease-oriented antitumor drug screening models. This project, begun as the DCT Lung Cancer Drug Discovery Project, was expanded to include other human and selected murine tumors. Essential to this effort is the collaboration of other DCT staff including the NCI-Navy Medical Oncology Branch (J. Minna). Over 30 different tumor lines have been acquired, expanded in cell culture, and cryopreserved in quantities adequate for characterization and initial experimentation. An automated growth inhibition assay suitable for large-scale screening is being developed. Actual drug screening is planned to begin in October, 1985. Of the compounds to be tested, 2,000 will be screened in vivo against P388 as well in order to provide comparative information on the two approaches to discovery of new antitumor drugs.

Development of Metastatic Human Tumor Models

This effort, conducted at FCRF, is closely integrated with the in vitro model development work described above. Metastatic strains of several human tumors have been selected from tissues (lung, liver, or lymph nodes) after implantation of human tumor cells by various routes. Cell strains from metastases of the aggressively growing LOX tumor appear particularly suited for experimental chemotherapy. Several lung tumors also appear promising for development of metastatic models.

Detailed Drug Evaluation and Congener Development

Treatment schedule and/or route dependency studies were completed for five clinical candidate drugs (13). Flavone acetic acid (NSC-347512) activity against mouse colon tumor 38 was greatest when a single massive dose was given. Multiple treatment regimens were less effective, probably related to the slow plasma clearance of the drug in mice. IP activity was retained when NSC-347512 was

given IV. Deoxyspergualin (NSC-356894) activity against SC implanted mouse leukemia L1210 was greatest on an intensive, intermittent schedule, q3hrs. q4 days. Experimental formulations were evaluated for six drugs. Results to date indicate that the formulations of the Sulfonate derivative, NSC-329680; Tetracarcin, NSC-333856; and the Anthrapyrazole derivative, NSC-349174 retained the activity of the bulk drug (13).

Studies to provide basic information on the cytotoxic and biochemical effects of seven antitumor agents being considered for development to clinical trial were initiated (14). Major emphasis was placed on elucidating the mechanism of action of the polyamine, Deoxyspergualin (DSG). Results have indicated that the cytotoxicity of DSG in vitro requires activation to the aldehyde by monoamine oxidase or enzyme(s) in calf serum. The properties of DSG appear similar to those of the endogenous polyamines, Spermine and Spermidine, which also require "activation" to aldehyde by calf serum enzyme(s) to inhibit growth of L1210 cells. Labelled precursor incorporation studies showed that the initial effect of activated DSG was inhibition of DNA synthesis.

In order to maximize the potential of lead compounds, new congener series were evaluated under specialized controlled experimental conditions based on the characteristics of the parent compound (12). Under this project special studies were conducted to answer questions arising during the development of a drug. During the past year, the contractor (12) received 470 new compounds for evaluation, including 68 synthesized under contract to DS&CB. Through congener development of the Isopropylpyrrolizine derivative, NSC-278214, a highly active agent which was dropped from development because of severe solubility and stability problems, a congener with equivalent P388 activity and increased aqueous solubility was found. NSC-369395, a congener of Camptothecin, with greater activity against L1210 has an amine substituent which should facilitate formulation. Special studies showed that Physostigmine, used in the clinic to alleviate the CNS toxicities of Spiromustine, did not reverse the anti-P388 activity of the latter.

Studies of melamines and triazenes, aimed at developing analogs with increased therapeutic efficacy were continued (15). Phase I clinical trial of Trimelamol, NSC-283162, is nearly complete. Twenty-nine patients have been treated by single dose rapid IV infusion every three weeks. Nausea and vomiting, lethargy and myelosuppression were observed toxicities. Nausea and vomiting were far less severe than previously observed with Pentamethylmelamine (PMM) and, unlike PMM, Trimelamol did not cause acute sedation. Evidence of antitumor activity was observed in five patients. Based on further evaluations of the phenyltriazenes, the contractor (15) selected 1-p-Carboxy-3,3-dimethylphenyltriazene (CB 10-277, the sodium salt of NSC-208107) for development to clinical trial. The compound was found to be metabolized in both rats and mice to the carboxylic acid glucuronide of the dimethyltriazene. No evidence was found for conversion to the monomethyl derivative; the monomethyl compounds are believed to be the active species of the carboxamido triazenes including DTIC. Presumably NSC-208107 does not require N-demethylation as it demonstrated antitumor activity against two human melanoma xenografts.

The Platinum Analog Working Group continued to coordinate the development of Tetraplatin, NSC-363812. This Cisplatin analog, discovered by DTP, retains antitumor activity against animal tumors with acquired resistance to Cisplatin, is sufficiently soluble and stable for parenteral use, and is less nephrotoxic than Cisplatin in rats (C. Litterst et al, DTP intramural laboratories).

National Cooperative Drug Discovery Groups

Eight applications were received in response to reissuance of the RFA for NCDDG Cooperative Agreements. Two were recommended for first year funding in FY 1985. The new five year awards are aimed at investigation of (a) topoisomerases as new therapeutic targets (W. E. Ross, University of Florida, P.I.); and (b) antireceptor monoclonal antibodies in cancer treatment (J. Mendelsohn, Memorial Sloan-Kettering Cancer Center, P.I.). These added to the two existing Groups (first year funding in FY 1984) will give us four NCDDG's comprising 20 laboratory projects in 14 academic, research, and industrial organizations. Anticipated FY 1985 funding for the four groups is over $2,000,000.

A distinguishing feature of Cooperative Agreements is the requirement for active participation of NCI staff during performance. DEB collaboration with the Group devoted to the synthesis of specific inhibitors of viral oncogene-encoded tyrosine specific protein kinases (V. Levin, University of California, San Francisco, P.I.) included identification of 284 candidate compounds which have been submitted to our antitumor screening program which have the desired structural characteristics of potential inhibitors. DEB collaboration with the Group (C. Porter, Roswell Park Memorial Institute, P.I.) devoted to the discovery of inhibitors of polyamine biosynthesis as potential antitumor agents included (a) preclinical antitumor evaluation of Difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, in combination with AraC based on reports that DFMO arrested L1210 cells and transformed SV3T3 cells in S-phase while mouse bone marrow and normal 3T3 cells were arrested in G1; and (b) presentation of Deoxyspergualin, a homolog of Spermidine, to the Group as a potential inhibitor of polyamine biosynthesis with demonstrated in vivo antitumor activity (see earlier discussion of DSG).

Publications by Staff

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5. Goldin, A., Venditti, J. M., and Geran, R.: The effectiveness of the anthracycline analog 4'-epidoxorubicin in the treatment of experimental tumors. Invest. New Drugs 3: 3-21, 1985.

6. Shoemaker, R. H., Wolpert-DeFilippes, M. K., Kern, D. H., Lieber, M. M., Makuch, R. W., Melnick, N. R., Miller, W. T., Salmon, S. E., Simon, R. M., Venditti, J. M., and Von Hoff, D. D.: Application of a human tumor colony forming assay to new drug screening. Cancer Res. 45: 2145-2153, 1985.

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Publications by Contractors

1. Corbett, T. H., Roberts, B. J., Leopold, W. R., Peckham, J. C., Wilkoff, L. J., Griswold, D. P., and Schabel, F. M.: Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57Bl/6 mice. Cancer Res. 44: 717-726, 1984.

2. Dumont, P, Van Der Esch, E., Jabri, M., Lejeune, F., and Atassi, G.: Chemosensitivity of human melanoma xenografts in immunocompetent mice and its histological confirmation. Int. J. Cancer 33: 447-451, 1984.

3. Griswold, D. P., Laster, W. R., Trader, M. W., and Dykes, D. J.: The preclinical scientific basis for adjuvant chemotherapy in breast cancer. In Senn, H. J. (Ed.): Recent Results in Cancer Research 96. Berlin, Springer-Verlag, 1984, pp. 1-7.

4. Houchens, D. P. and Ovejera, A. A.: Heterotransplantation Models for Screening - Current Status and Future Prospects. Principles of Cancer Chemotherapy (in press).

5. Simpson-Herren, L.: Autographic Techniques for Measurement of the Labeling Index. Techniques for Analysis of Cell Proliferation (in press).

TABLE 1

CONTRACTS MANAGED BY DER AND FUNDING LEVELS, FY 1985

In Vivo Screening	$3,140,458
1. Southern Research Institute (4-7646)	780,033
2. Mason Research Institute (4-7647)	767,500
3. Battelle-Columbus Laboratories (4-7648)	661,911
4. IIT Research Institute (4-7567)	780,802
5. Institut Jules Bordet, Belgium (5-7645)	150,212
In Vitro Screening (HTCFA)	$ 661,875
6. UCLA (5-7710)	187,079
7. Mayo Foundation (5-7711)	216,015
8. University of Arizona (5-7662)	258,781
New Model Development	$ 680,000
9. PRI-FCRF	
In Vitro Human Tumor Cell Lines	440,000
Discovery of New Biological Models	90,000
A&E Phase I Construction	150,000
10. University of California, San Diego (3-7568)	0*
Tumor Quality Control	$ 301,725
11. Southern Research Institute (4-7581)	301,725
Detailed Drug Evaluation	$1,076,504
12. Southern Research Institute (3-7552)	286,374
13. Southern Research Institute (4-7580)	611,630
14. Southern Research Institute (4-7615)	178,500
15. Institute of Cancer Research, England (4-3736)	0+
Support Services	$ 216,366
16. S.B.A. Biotech (3-7558)	216,366
TOTAL	$6,076,928

*Terminated August 31, 1985.
+Terminated June 30, 1985.

TABLE 2

COMPOUNDS SELECTED FOR DEVELOPMENT
APRIL 1, 1984 - MARCH 31, 1985
(DECISION NETWORK 2A)

NSC No.	Name	Assignment Date	Selection Basis*
95678	3-Hydroxy-2-formylpyridine thiosemicarbazone (3-HP)	84-06-12	(Project to Review Old Drugs)
291643	Pyrimidineacetaldehyde derivative	84-08-14	IP L1210 Leukemia IP M5076 Sarcoma SRC MX-1 Mammary Tumor Xenograft
326231	L-Buthionine Sulfoximine	84-06-12	(Radiosensitizer Chemosensitizer)
339004D	(Discreet)	84-06-12	(Human Tumor Colony Forming Assay)
339736D	(Discreet)	84-06-12	(Human Tumor Colony Forming Assay)
343513	Dihydrolenperone	84-04-10	(Human Tumor Colony Forming Assay)
361456D	(Discreet)	84-12-05	IP B16 Melanoma IP L1210 Leukemia IP M5076 Sarcoma SRC MX-1 Mammary Tumor Xenograft
364372	Elactocin	84-08-14	IP B16 Melanoma IP M5076 Sarcoma

* Tumor panel systems in which a compound (non-formulated product) has demonstrated sufficient in vivo antitumor activity to warrant consideration for development to clinical trial (DN 2 activity). Information in parenthesis indicates reason for passing DN 2A other than in vivo activity in a tumor panel model.

ANNUAL REPORT OF THE ANIMAL GENETICS & PRODUCTION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

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, tumor panel, and Toxicology Program; (2) other NCI research contracts (DCCP, DCBD, etc.); (3) NIH and FCRF Intramural Programs; (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 potentially 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 rodents 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, isozyme electrophoretic studies, etc., as needed.

3. To provide metastatic human/murine tumor models which offer potential for usage on 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 accreditation requirements

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

6. To assist the DTP in upgrading extramural contractor usage laboratory facilities and management to the extent that experimental animals can be maintained in the same pathogen free status as received from the AG&PB.

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

5. 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 1985

PRIMARY GENETIC CENTERS (4)	$4,703,000
Supply breeding nucleus for the animal program and athymic mice for drug evaluation.	
RODENT PRODUCTION CENTERS (5)	767,000
Large-scale production of inbred mice and nude mice under both conventional and barrier controlled environment.	
HYBRID MOUSE PRODUCTION CENTERS (6)	4,000
Supply hybrid mice for the screening program. These contracts terminated January 31, 1985 and were not renewed.	
DIAGNOSTIC & HISTOCOMPATIBILITY PROJECTS (8)	901,000
To monitor animal health and genetic integrity.	
DEVELOPMENT OF STANDARDS & GUIDELINES (1)	34,000
For animal care and breeding.	
MAINTENANCE OF FROZEN TUMOR BANK (1)	300,000
FREDERICK CANCER RESEARCH FACILITY (2)	3,308,000
CENTRALIZED REDERIVATION (1)	105,000
Rederiving new starts from the NIH Repository into associated flora status.	
TOTAL	$10,122,000
Less Reimbursements (Including Grantee Collection)	3,192,000

ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

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 22 contracts with a combined annual budget of approximately 6.9 million dollars.

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 Pharmacology and Experimental Therapeutics are assigned to the formulation 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, two visiting visiting fellows 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

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 and pilot plant production of various quantities of bulk substances intended for tumor screening panels, preclinical toxicology and pharmaceutical manufacture of investigational products for clinical evaluation.

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 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 34 man years. Additionally, two radiosynthesis laboratories are supervised by the Chemical Resources Section with a combined annual effort of 5.5 man years.

2. Analytical and Product Development Section

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.

Presently, the Section's analytical chemist supervises three analytical contracts representing a combined 18 staff year effort and a shelf life surveillance contract for clinical products involving an annual three and one-half staff year effort. These contractors have the expertise to chemically characterize a very structurally diverse group of chemicals. These contractors are also responsible for the development and application of stability-indicating methods for all new drug substances.

The dosage form development component of the Section is responsible for conversion of bulk chemical into a pharmaceutical product suitable for clinical use. Essentially all products are developed for intravenous use. About one-half of the drugs do not exhibit adequate solubility or stability and some form of pharmaceutical intervention is required. Standard approaches (salts, solvents and surfactants) are initially evaluated. Emphasis is also given to evaluation of newer techniques to improve solubility or stability (emulsions, prodrugs and complexation). The developed dosage form is evaluated for chemical content, antitumor activity in the rodent screen and feasibility to be manufactured on production scale.

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.

Analytical data developed on new investigational compounds is assembled and published in a book entitled "NCI Investigational Drugs - Chemical Information." This text contains stability-indicating methods, spectral data, approximate solubility and stability data, and other appropriate information on a large number of agents. The publication is distributed to investigators throughout the country.

3. Clinical Products Section

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 2.0 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. 85-2141) which is updated periodically. During this reporting period, over 4,000 issues were distributed.

Goals and Accomplishments

The Pharmaceutical Resources Branch has successfully accomplished its objectives in providing high quality bulk chemicals and pharmaceutical products to the various Programs in the Division of Cancer Treatment. During this reporting period, the prep lab contractors prepared over 181 compounds totaling more than 100 kilograms. Examples of bulk pharmaceutical substances which when delivered for formulation included: Carboplatin (NSC-241240); 4-Ipomeanol (NSC-349438); Methotrexate (NSC-740); Ara-AC (NSC-281272); Fludarabine (NSC-312887); and L-Buthionine-S, R-sulfoximine (BSO) (NSC-326231). The Clinical Products Section directed the contract production of more than 450,000 injectable units and more than 880,000 oral dosages for clinical distribution.

The pharmaceutical research and development activities were also quite successful in completing development of nine formulation assignments including parenteral products of Tetraplatin (NSC-363812); Pyrazole (NSC-45410); 4-Ipomeanol (NSC-349438); Anthrapyrazole (NSC-349174); and others.

Many of the new agents submitted for development present formulation problems due to poor aqueous solubility and/or stability. In view of these problems, the Pharmaceutical Resources Branch submitted a Request for Proposal to the Small Business Incentive Research (SBIR) Program for research proposals in the area of Novel Drug Formulation and Delivery Systems. Nine proposals were received and multiple awards are expected. An additional Request for Proposal was submitted under this same Program for a Computerized Pharmaceutical Tracking System which is expected to provide historical and control data on all products under investigation. Seven proposals were received for the project and multiple awards may be made.

Also during this reporting period, the Clinical Products Section became increasingly involved with the management of acquisition and distribution of biological products for the Biological Response Modifiers Program (BRMP). More than 20 BRMs have been received under this project and many more are expected in the next several months. The products received during this period included Interleukin-2; Thymosin; Alpha-Interferon; Lymphoblastoid Interferon; and Monoclonal Antibodies. The Pharmaceutical Resources Branch was also involved in providing pharmaceutical support for the Division of Cancer Prevention and Control (DCPC).

During this reporting period, the Pharmaceutical Resources Branch participated in the newly formed NCI/European Collaborative Program which is an experimental scientist exchange program. Two scientists, Dr. John Slack from the University of Aston in Birmingham, England and Ms. Pamela Warrington, Senior Oncology Pharmacist from Edinburgh, Scotland, spent four weeks and three weeks, respectively, with PRB staff learning pharmaceutical aspects of investigational drugs.

Publications by Staff

1. Cheung, W. Y., Vishnuvajjala, B. R., and Flora, K. P.: Stability of cytarabine, methotrexate sodium, and hydrocortisone sodium succinate admixtures. Am. J. Hosp. Pharm. 41: 1802-1806, 1984.

2. Cheung, W. Y., Vishnuvajjala, B. R., Morris, N. L., and Flora, K. P.: Stability of azacitidine in infusion fluids. Am. J. Hosp. Pharm. 41: 1156-1159, 1984.

3. Davignon, J. P. and Cradock, J. C.: Pharmaceutical aspects of antitumor agents. Pharmaceutisch Weekblad 46: 1144-1150, 1984.

4. McCarthy, L. E., Flora, K. P., and Vishnuvajjala, B. R.: Disparaties in the antiemetic and behavorial actions of delta-9-tetrahydrocannabinol and its 11-OH-metabolite in the cat. Res. Comm. Sub. Abuse 5: 103-114, 1984.

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Willette, R. E. (Eds.): The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects. New York, Academic, 1984, 909 pp.

6. Vishnuvajjala, B. R. and Cradock, J. C.: Compatibility of plastic infusion devices with diluted N-methylformamide and N,N-dimethylacetamide. Am. J. Hosp. Pharm. 41: 1160-1163, 1984.

7. O'Dwyer, P. J., Shoemaker, D. D., Plowman, J., Cradock, J. C., Grillo-Lopez, A., and Leyland-Jones, B.: Trimetrexate: a new antifol entering clinical trials. Investiga. New Drugs 3: 71-75, 1985.

8. Perlin, E., Smith, C. G., Nichols, A. I., Almirez, R., Flora, K. P., Cradock, J. C., and Peck, C. C.: Disposition and bioavailability of various formulations of tetrahydrocannabinol in the rhesus monkey. J. Pharm. Sci. 74: 171-174, 1985.

9. Trissel, L. A., Davignon, J. P., Kleinman, L. M., Cradock, J. C., and Flora, K. P.: NCI Investigational Drugs - Pharmaceutical Data 1985. NIH Publ. No. 85-2141, 1985, 192 pp.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-CM-03584-13 PRB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James C. Cradock	Head	A&PDS	PRB	NCI
Other:	Karl P. Flora	Chemist	A&PDS	PRB	NCI
	Babu R. Vishnuvajjala	Visiting Assoc.	A&PDS	PRB	NCI
	Yuen Cheung	Visiting Fellow	A&PDS	PRB	NCI

COOPERATING UNITS *(if any)*

Dr. Charles Litterst, DTP, DCT, NCI

LAB/BRANCH

Pharmaceutical Resources Branch

SECTION

Analytical and Product Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

This project describes the activities of the formulation laboratory of the Pharmaceutical Resources Branch. These studies are directed toward resolving problems in the intravenous delivery of antitumor agents and involve methods to evaluate and then improve drug solubility and stability. Pharmaceutical aspects of the new platinum analogue, tetraplatin, were examined. Stability-indicating HPLC methods were developed and applied to an evaluation of this compound under a variety of conditions and a freeze dried dosage form was developed.

The safety of certain antitumor agents has been a topic of considerable concern to personnel involved with the handling and administration of these compounds. Procedures to decompose (two-three log reduction) fourteen compounds have been developed using simple procedures: base, hydrogen peroxide, and/or heat. These compounds represent most of the chemical categories of clinically active antitumor agents.

Methods to improve drug solubility have also been evaluated. Several water soluble prodrugs of camptothecin have been prepared and evaluated in suitable tumor models. The N,N-diethyl glycine derivative exhibited activity and potency similar to that demonstrated by camptothecin. Also the influence of the polymer, poly l-methionine sulfoxide on the solubility of several antitumor agents was studied.

PHS 6040 (Rev. 1/84)

GPO 914-918

1. Stability, Formulation Development and Pharmacology of Tetraplatin (Cheung, Vishnuvajjala, Flora, Cradock and Litterst).

   Tetraplatin (NSC-363812) is a new antitumor platinum derivative under development in the Developmental Therapeutics Program. The rationale for pursuing the eventual clinical trials of this complex lies mainly in its activity in cisplatin resistant L1210 murine tumor systems. The predecessor to this compound was NSC-271674 a DACH Pt with a tricarboxybenzene leaving group. This compound was not amenable to analysis by any of the usual analytical assay techniques. In addition, there was substantial evidence that the compound was extremely unstable in aqueous solution. Tetraplatin, however, can be assayed by HPLC and characterized by other standard analytical techniques. Although aqueous instability is apparent for tetraplatin, it is possible to significantly inhibit the decomposition of the intact drug by adjusting the chloride content of solutions. A stability-indicating HPLC method was developed to monitor the drug in various solutions and infusion fluids. The influence of pH on drug stability was studied in drug solutions (0.9 mg/ml) buffered at pH values between 3.0 and 7.0. The rate of decomposition of tetraplatin increases as the solution pH decreases. Solutions of tetraplatin in distilled water, Sterile Water for Injection and 5% Dextrose Injection show concentration-related differences in the rate of decomposition. The magnitude and time course of these differences has been somewhat erratic and irreproducible. However, simultaneous monitoring of chloride ion release using a chloride selective electrode in conjunction with the HPLC assay of the same solution indicate that the observed differences are real and are not an artifact of the chromatographic assay procedure. Studies on the stability of these solutions are ongoing.

   The stability of tetraplatin is greatly enhanced in chloride containing solutions. Drug concentration effects on stability are less marked. Drug concentrations of 0.1 - 5 mg/ml in 0.9% sodium chloride show less than 10% decomposition in 6 hours in all situations and are suitable for administration by either infusion or direct intravenous injection. Tetraplatin can be freeze dried from solutions containing sodium chloride and mannitol.

   Dr. Litterst and his colleagues are evaluating the pharmacology of tetraplatin in rodents. We are collaborating on a method to detect drug and metabolites in biological samples. This procedure will use HPLC separation and analysis of the eluates for platinum by atomic absorption. The ultraviolet absorption of tetraplatin is inadequate at concentrations found in biologic samples to use conventional HPLC separation and UV detection. Preliminary protein binding studies have been conducted and other plasma level measurements are being pursued.

2. Methods for the Forced Decomposition and Decontamination of Residues of Formulated Antitumor Agents (Cheung, Vishnuvajjala, Cradock and Flora).

The Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute is responsible for producing and supplying formulated antitumor agents for clinical studies. In recent years some medical personnel have expressed concern regarding the handling and disposal of these compounds which may be potential mutagens or carcinogens.

We have recently evaluated a number of drugs in hopes of providing simple procedures to rapidly decompose the drug entity as it exists in the dosage form. It is hoped that these procedures will aid in the safe and efficient disposal of these agents. Also these procedures may be useful in the decontamination and clean-up of inadvertent spills in hospital and clinic settings.

Results have been obtained on fourteen drugs. Decomposition is forced by the use of sodium hydroxide, hydrogen peroxide or heat or combinations of the above. The disappearance of drug was monitored by high performance liquid chromatography (HPLC) in all cases. The target for each drug was to force decomposition to the point that no HPLC peak for the intact drug was observed (2-3 log reduction). Using these procedures the following drugs have been decomposed: Adriamycin, Daunorubicin, Aclarubicin, Amsacrine, Menogaril, Mitomycin, Dactinomycin, Diaziquone, PCNU, BCNU, Azacitidine, Melphalan, and Mitoxantrone. Ames testing will be performed on each of the intact formulations and on the decomposed solutions to determine if the chemical treatments are associated with a comparable loss of biologic activity.

3. Camptothecin (Drs. Vishnuvajjala, Cheung, Flora and Cradock).

Camptothecin is a poorly water soluble natural product that is very active in experimental tumor models. The sodium salt of the lactone is active but at tenfold higher doses. Several prodrugs of camptothecin were prepared that have greater water solubility but retain the lactone ring.

The new derivatives involve esterification with substituted amino acids of the hydroxyl group at position 20. Hydrochloride salts were then prepared from the bases. Five prodrugs were prepared, characterized chemically and found to be the expected structure with a purity by TLC and HPLC of > 95%. Solubilities ranged from 2 to 8 mg/ml and were adequate to deliver the projected doses. Antitumor data on the N,N diethylglycine derivative indicates that the activity is comparable to camptothecin at similar doses. The other derivatives are substantially less potent than camptothecin.

A HPLC method was developed to determine concentrations of the N,N-diethylglycine derivative in plasma. Studies to evaluate the rate of reversion of the ester to camptothecin were conducted in out-dated human plasma and in a solution containing a non-specific esterase. A paraben ester was used as a positive control in the non-specific esterase solution study. No significant ester hydrolysis was noted after incubation at 37° for 1 to 2 hours in either matrix. A small study in mice is planned to see if any significant conversion takes place in vivo.

Several small projects were also carried out during this period. Formulation work on the antitumor agent diaziquone was completed in this laboratory several years ago using 5% dimethylacetamide (DMA) to achieve the required solubility. Diaziquone has subsequently undergone extensive clinical trial mostly by the intravenous route but has also been tested intrathecally. Recently Dr. Poplack, COP, NCI, noted elevations of myelin basic protein in humans receiving diaziquone to treat CNS leukemia and in monkeys injected with the DMA containing vehicle. The product was reevaluated in an attempt to reduce or eliminate this solvent. The same drug solubility could be achieved by reducing the DMA content by one-half or substituting DMSO at one-fifth the amount. However, adequate solubility could not be achieved in the absence of an organic solvent. The DMSO formulation will likely be evaluated intraventricularly in monkeys.

Poly l-methionine sulfoxide was reported by others to improve the water solubility of several compounds. The influence of this polymer on the solubility of several poorly water soluble antitumor agents was evaluated. Pharmaceutically significant solubility increases have not been observed.

Publications

1. Cheung, Y.W., Vishnuvajjala, B.R., Morris, N.L. and Flora, K.P.. The Stability of Azacitidine in Infusion Fluids. Am. J. Hosp. Pharm. 41: 1156-1159 (1984).

2. Cheung, Y.W., Vishnuvajjala, B.R. and Flora, K.P.. Stability of an Admixture of Cytarabine, Methotrexate and Hydrocortisone Sodium Succinate in Infusion Fluids. Am. J. Hosp. Pharm. 41: 1802-1806 (1984).

3. Tabibi, S.E. and Cradock, J.C.. Stability of Melphalan in Infusion Fluids. Am. J. Hosp. Pharm. 41: 1380-1382 (1984).

4. Vishnuvajjala, B.R. and Cradock, J.C.. Compatability of Plastic Infusion Devices with N-Methylformamide and Dimethylacetamide Containing Solutions. Am. J. Hosp. Pharm. 41: 1160-1163 (1984).

5. McCarthy, L.E., Flora, K.P., Vishnuvajjala, B.R.. Disparities in the Antiemetic and Behavioral Actions of Delta-9-Tetrahydrocannabinol and its 11-OH Metabolite in the Cat. Res. Comm. Sub. Abuse. 5:103-114 (1984).

6. Ray, G., Crook, M., West, N., Kwoka, M., Rehagen, G., Cox, J., Murrill, E. and Flora, K.. Comparison of the Analysis of Delta-9-Tetrahydrocannabinol Capsules by High-Performance Liquid Chromatography and Capillary Gas Chromatography. J. Chromatogr. 317:455-462 (1984).

7. Perlin, E., Smith, C.G., Nichols, A.I., Almirez, R., Flora, K.P., Cradock, J.C. and Peck, C.C.. Disposition and Bioavailability of Various Formulations of Tetrahydrocannabinol in the Rhesus Monkey. J. Pharm. Sci. 74:171-174 (1985).

ANNUAL REPORT OF THE TOXICOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

The mission of the Developmental Therapeutics Program centers on the discovery and preclinical development of agents with clinical anticancer potential. Investigations focusing on the hazards of antineoplastic compounds to healthy organs in intact experimental animals are the final steps in the preclinical stages of new drug development. Such investigations comprise the primary responsibility of the Toxicology Branch. Toxicology studies designed to meet this responsibility involve three major objectives:

1. Determination and safety assessment of an initial dose for clinical trial.

2. Determination of primary organ systems adversely affected by drug administration.

3. Determination of the reversibility of the adverse effects.

Data generated from studies on each new drug and evaluated in light of potential human toxicity comprise the major portion of the evaluable information required by the Food and Drug Administration for an Investigational New Drug Application.

During this fiscal year the direction of the Toxicology Branch has shifted from an unidirectional course to two highly integrated paths. The first path continues to be the elucidation of the potential adverse effects of new anticancer agents. The second path is the acquisition and use of pharmacokinetic information to reliably extrapolate toxic effects across species by relating plasma drug levels (peak and steady state) to the appearance and severity of toxicity. Integration of these two courses permits a more rational evaluation of the role of schedule dependence in efficacy of drug as well as in development of toxicity.

In the main, the Branch achieves its primary responsibility through the operation and management of a prime contract in which the qualitative and quantitative toxicological profiles of antitumor drugs and modalities are determined in experimental animals. For 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 guidelines using mice, rats and dogs are followed to determine the initial dose for Phase I clinical trials, to verify safety of the initial clinical dose and to elucidate specific target organ toxicity and its reversibility. Task II studies are concerned with limited evaluations of drugs. 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 studies. 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.

An extramural contract effort in pharmacology and pharmacokinetics, a Master Agreement arrangement, has been functional for two years. The majority of the preclinical pharmacology data used in our analysis of kinetics and continuous delivery is developed under this project. Protocols are divided into three phases, with phases 2 and 3 usually performed in mice.

1. Analytical Methods Development.

2. Plasma elimination following a bolus dose.

3. Plasma pharmacokinetics during continuous drug delivery.

Information from these studies form the basis for similar studies in dogs. All data are then integrated with the toxicity studies to provide the cornerstone for drug characterization which more closely meets clinical needs.

The Division of Cancer Treatment maintains a master file with the FDA which contains toxicity testing protocols for antitumor agents. These protocols set forth jointly agreed to procedures for animal toxicity testing of antineoplastic drugs. Data from studies conducted under the protocols are accepted for regulatory purposes in INDA approval. The Toxicology Branch has amended the protocols to accurately reflect newer methods and techniques. Additionally, individualized protocols (drug specific) are routinely developed to account for agent specific chemical and/or physical properties. During the past year, the Branch has collected and evaluated pharmacokinetic data in addition to toxicity data in an effort to understand the in vivo behavior of new agents related to toxic findings. New guidelines, dependent on drug characteristics, for pharmacokinetic studies and continuous delivery procedures in mice and dogs have been prepared and submitted to the Food and Drug Administration for inclusion in the Master File.

Data on the following agents have been, or are anticipated to be, presented to the Decision Network Committee in FY'85.

Flavone Acetic Acid	NSC-347512
Merberone	NSC-336628
Deoxyspergualin	NSC-356894
Pibenzimol	NSC-322921
Triazine Antifol	NSC-127755

Flavone Acetic Acid should be an exciting and important addition to the anti-cancer drug arsenal. The agent appears to be exceptionally active against solid tumors of endothelial origin, notably colon cancers in mice. The drug is relatively non toxic to dogs at doses, and concomitant blood levels, which are very effective in murine tumor systems. Sufficient preclinical data are available to suggest a clinical dose escalation scheme based on pharmacokinetic

parameters. Merberone and Deoxyspergualin are active in experimental leukemias and lymphomas. Pharmacokinetic data suggest that efficacious plasma levels of these two drugs can be achieved and maintained in the absence of life-threatening toxicity.

Toxicology studies have been, or are anticipated to be, initiated in FY'85 on the following compounds:

4-Ipomeanol	NSC-349438
Azacytosine Arabinoside	NSC-281272
Anthrapyrazole	NSC-349174
Tetraplatin	NSC-363812
Buthionine Sulfoxamine	NSC-326231
Discreet Agent	NSC-339004D
Hexamethylene Bisacetamide (po)	NSC-95580

Studies on these agents likely will be completed during FY'86 and will provide a complete picture on their toxic effects and pharmacokinetic behavior using the integrated approach developed over the past two years.

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5. Tong, S., Hirokata, Y., Litterst, C.L. and Gram, T.E.: Interaction of the oncolytic drug, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) with the mixed function oxidase system in rats. Chem. Biol. Interact. 49: 105-119, 1984.

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10. Okine, L.K., Goochee, J.M., and Gram, T.E.: Studies on the distribution and covalent binding of [^{14}C] 1,1-dichloroethylene in the mouse: effects of various pretreatments on covalent binding in vivo. Biochem. Pharmacol. in press.

11. Okine, L.K. and Gram, T.E.: Protection by methylprednisolone against butylated hydroxy-toluene-induced pulmonary damage and impairment of microsomal monooxygenase activity in the mouse: lack of effect on fibrosis. Exp. Lung Res. in press.

12. Gram, T.E.: Metabolism of drugs. In Craig, C.R. and Stitzel, R.E. (Eds.): Modern Pharmacology. Boston, Little, Brown & Co., in press.

13. Okine, L.K.N. and Gram, T.E.: Drug absorption and distribution. In Craig, C.R. and Stitzel, R.E.(Eds.): Modern Pharmacology. Boston, Little, Brown & Co., in press.

Contractors

During this year contractors and subcontractors to the Toxicology Branch presented 6 papers or abstracts which involved NCI support.

ANNUAL REPORT OF THE INFORMATION TECHNOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

A fully operational version of the Drug Information System (DIS) was installed in March 1985. That allowed the NCI contract with the Chemical Abstracts Service (CAS) to be terminated the following month. The NCI-CAS contract had been in existence for a dozen years and its end, with the start-up of the DIS, marks a turning point in computer use in DTP. The DIS represents a consolidation of most of the disparate computer systems used by the DTP; it permits interactive control of acquisition, shipping, inventory and testing of chemicals. The major DIS databases are self-updating and the updating proceeds automatically at pre-determined dates and times. All output from the DIS is directed to a high-speed laser printer in the Landow Building. This printer is capable of graphical and non-graphical printing and represents a major advance over the earlier procedures which required the use of several different printers and which, as a result, could take hours or even days to complete. Since the DIS was released, it has logged tens of thousands of transactions. Numerous minor problems have been encountered and resolved and many of the important DIS procedures have been greatly optimized - a process which has been aided significantly by the installation at DCRT of a third KL-10 processor in the DEC System 10 computer.

Meanwhile, the ITB supports the information needs of the DTP by providing data to the program in many ways. With DTP's access to data supported by the DIS, the requirements for reports which have to be generated by ITB staff are actually decreasing. The ITB staff is, therefore, able for the first time to devote its attention to collecting and organizing much data that is ancillary to the screening work, e.g. flow of mice and data pertaining to plant collection. During this year, furthermore, there has been increasing emphasis on the use of graphics to represent the complex data used by DTP. In this connection, several distinct programs have been written for the display of screening data and some of these are now being used regularly.

Finally, in an exciting new excursion, the Branch is involved in the design of a computer system which is to be central to the new cell line project which the DTP is embarking upon. The new data system will run on a series of independent local microprocessors, controlled by and reporting to the DIS. In this project the throughput requirements are very high. Accordingly, a commitment has been made to robotics, which promises to deliver such a high volume of tests.

Office of the Chief, ITB

This Office supervises and coordinates the activities of the two Sections of this Branch, directs the progress of the 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. Drug Information System.

After the first release of the DIS on March 1, 1985, careful monitoring of the system for accuracy and performance began. Very few inaccuracies (data errors) have been found; those that were discovered have been corrected and the DIS is thought currently to be essentially error-free. The initial slow response encountered was traced to two problems; computer overloading and inadequate optimization in some parts of the programs. The first of these problems was essentially eliminated at mid-April with the expansion of the DEC-10 computer by a factor of over 50%. Work on optimization still continues, but the major difficulties were resolved during May, and since then, response time has not been cited as a serious problem.

The interfacing of the high-speed laser printer to the computer system has been completed, but handling of graphics by this printer is not yet trouble-free. In the online environment, the electronic balances are working well and the bar code technology is proving to be a major strength of DIS. An extensive effort to provide systems level documentation of the DIS has begun and should be completed before the end of 1985.

Support for users of the DIS is provided by ITB. Five different User's Manuals have been written and published and two more are in preparation. In addition, a total of over 400 online "HELP" messages have been written and made available to online users. Finally, during May and June, a series of 2-hour seminars on use of the DIS was presented to DTP staff.

Future work on the DIS will be directed at remaining DTP requirements such as animal and plant resource files, the toxicology file and the file of bulk chemicals prepared under Good Manufacturing Practices.

2. Computer System for the Cell Line project.

The DTP has begun a major new initiative which involves the testing of chemicals in vitro for selective cytotoxicity towards specific cell lines. It is hoped ultimately to be able to complete as many as a million such tests per year. Such a level of testing mandates strong computer support. Accordingly, a system, centered upon a group of microprocessors in the laboratory has been designed. These processors will accept strategic commands from the DIS and return semi-processed data to the DIS. The microprocessors will achieve much of the necessary work by issuing commands to robots, which will do a great deal of the actual sample handling, and can readily do so under sterile conditions. A prototype version of this system should be running by the end of 1985.

3. Automation of Literature Surveillance.

Literature surveillance, a manpower-intensive task, is currently carried out by two contractors to the DTP. During this year, the ITB monitored the entire literature for two months, comparing the results of a manual review against one performed by a computer, using a sophisticated model. The results demonstrated that the computer is considerably cheaper and more effective than a manual reviewer. It reviews many more published compounds and flags active compounds at a much higher rate (18% versus 3%). On the other hand, the computer cannot easily deal with non-algorithmic constraints.

4. Natural Products File.

The "Berdy File" of antibiotics is a useful resource whose value has always been compromised by the absence in the file of chemical structures. During this reporting period ITB, taking advantage of the programs developed in the Branch, has begun systematically to add structural data to the Berdy File. Some 6,000 structures of compounds in this file are known; over half of these have now been entered and completion of this file is scheduled for late 1985. It will then become searchable by structure and substructure as well as by other information types (micro-organism, author and so on) and should prove to be a valuable resource to DTP.

5. Automation of ELISA Assays.

In connection with the AIDS program, LTCB conducts a large number of ELISA and ELISA-like assays each year. The large amounts of data so produced proving to be a problem, ITB designed, developed and installed a data system on the Apple II computers in LTCB. This system works very well and has greatly assisted the LTCB in maintaining the flow of samples through their analytical laboratories.

6. ITB Reports.

This year, ITB has prepared, written and distributed 6 Reports dealing with subjects ranging from computer coding of tautomeric structures to new Investigational New Drug Applications filed by DCT during the year.

Biological Information Section, BIS

1. Screening Database.

Maintenance of this database has proceeded very smoothly during the year. Files resulting from the annual update in March were passed to the DIS and, for the first time, were made searchable online by the DIS. Data from the *in vivo* screening as well as from the HCTFA flow uneventfully into the Biology database. Retrieval of these data in a variety of forms is provided for and thousands of such retrievals are carried out each year. Many of the retrievals are completed by ITB staff themselves, using systems provided by ITB.

2. Data Retrieval and Report Generation.

The standard support for DTP data retrieval has been provided. A total of 324 Screening Data Summary reports have been run during the year - the number of NSC Numbers in such a report can range from one to several hundred. In addition to such routine support, a detailed correlation of preclinical and clinical activities for about 100 drugs was completed for the January 1985 review of the screening program. Another study which was completed, compared the data from *in vivo* testing to that from *in vitro* testing for some standard agents. This was done as a preliminary to the Cell Line project. A report containing detailed chemistry and summary biology data for 1300 active, open compounds was produced in October 1984. These compounds, no longer under active investigation by NCI, are of possible value to other organizations interested in development of antitumor agents.

3. Microprocessors.

Among the many strengths of the microprocessor is its ability to serve as a very powerful communications node. The IBM PC which the BIS acquired this year is used very heavily in this manner. It is frequently necessary to move several thousand NSC Numbers and records from one computer to another - for instance from the DCRT IBM system to a private sector computer for literature searching or to the IBM PC in the Brussels liaison office. Such transfers are now done with the PC. It can be connected first to one, then to the other computer, or alternatively, it can be used to collect data and write it to a floppy disk which can be mailed to Brussels.

4. Animal Utilization.

A computer system has been established which monitors the flow of mice from the suppliers to the screeners. Each screening laboratory now reports regularly (along with screening data) the numbers of mice ordered, received, used, and so on. These data are collected by programs which process them and automatically produce, upon a monthly basis, curves of these data for each strain/screener combination. This system is now working well and has permitted DTP staff to be alerted to and to resolve some major problems in the utilization of mice by the DTP. A parallel program handles the cost informationn of the mouse supply program; this is provided to the Animal Genetics and Production Branch as a management tool.

5. Plant Collection Database.

A system has been developed to manage the needs of the increasing effort in plant collection of the Natural Products Branch. Collectors in the field will enter data concerning their collection activities into a locally operated IBM PC. These data will be copied to a disk which will be mailed to DTP. Programs have been written to read such a disk and to use the data to update the "Plant Header File".

Chemical Information Section, CIS

1. Input of Chemical Structures.

The structure-input program that runs on a light-pen driven PC has been optimized and enhanced. It now can accept a large structure in about one minute and, as such, is no longer the rate-limiting step in data entry. Accordingly, this program is now in routine production use by the acquisitions contractor. A newer, radically different program which runs on an IBM PC has been completed. It is based upon a markedly different philosophy and, as hoped, it allows one to enter structures much more rapidly - as little as a few seconds suffices in many cases. The new program has been patented in behalf of the U.S. Government; enquiries as to licenses have already been received, and the massive data input to which it seems to lend itself is currently being investigated. A good deal of time has been devoted to the implementation of a number of graphical approaches to the presentation of screening data. This has led to "flowers", bar-graphs, and survival curves. All three of these techniques have been made available to the general user via interactive programs and the latter two are finding some measure of acceptance.

2. Output of Chemical Structures - Computer Graphics.

The procedures laid down within the DIS for the input, storage, and presentation of chemical structures are now working, apparently successfully. The last link in this chain is the printing of the structure on the laser printer and this step proved to be quite difficult, because of the number of useful variables such as the size of the structure, its orientation (and that of the accompanying text) on the page and the desirability of access to different type fonts (italics, greeks, bolds etc). Once these questions had all been resolved and structure drawing had become more routine, the more general problem of handling by the printer of generalized graphics was addressed. Work in this area, which is still going on, is aimed at printing a wide variety of graphics such as plots, bar-graphs, flow-charts or logos and signatures.

Publication:

Kerekes, P., Sharma, P. N., Brossi, A., Chignell, C. F., and Quinn, F. R.: Synthesis and Biological Effects of Novel Thiocolchicines. 3. Evaluation of N-Acyl Deacetylthiocolchicines, N-Alkoxycarbonyl-Deacetylthiocolchicines and O-Ethyl Demethylthiocolchicines. New Synthesis of Thiodemecolcine and Anti-leukemic effects of 2-Demethyl- and 3-Demethylthiocolchicine. J. Med. Chem., in press.

ANNUAL REPORT OF THE EXTRAMURAL RESEARCH AND RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

Description

The Extramural Research and Resources Branch is responsible for the administration of preclinical grant-supported research leading to the development of effective anticancer 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, comparative pharmacology and toxicology, and mechanism of drug action. As new findings and significant developments occur, appropriate changes are made in programmatic emphasis and support in each of its major research categories. 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. Projects of high programmatic interest are funded through the special exceptions procedure provided by the NCI Executive Committee, while proposals with objectives not of immediate relevance to drug development are transferred to other institutes or to more appropriate programs within the National Cancer Institute.

During Fiscal Year 1985, the Branch supported 283 research projects totalling $38.1 million dollars. The distribution of projects among the major categories shown in the following table is in accordance with the need and current priorities for research in the respective drug development area.

BIOCHEMISTRY AND PHARMACOLOGY PROGRAM
BY SUB-CATEGORY
FY 1985

	Number of Grants	Total Amount (Thousands)
SYNTHESIS & CHEMISTRY	83	$ 9,464
NATURAL PRODUCTS	31	3,538
SCREENING & EXPERIMENTAL THERAPEUTICS	32	3,507
COMPARATIVE PHARMACOLOGY	19	1,824
OTHER PRECLINICAL ASPECTS	8	763
MECHANISM OF ACTION	103	11,708
PROGRAM PROJECTS	7	7,272
TOTAL	283	$ 38,076

Significant Recent Results

Chemistry of Platinum Anticancer Drugs

The clinical effectiveness of cis-diamminedichloroplatinum (II), cis-DDP, against testicular, ovarian and bladder tumors has encouraged research into its mechanisms of antitumor activity, and into its synergistic reactions with other drugs in combination chemotherapy protocols. Pretreatment of DNA restriction fragments with cis-DDP modulated and activated the sequence specific cleavage by bleomycin. Cis-DDP may modify DNA structure in a way that facilitates action by another agent. By chemically linking a cis-$PtCl_2$ fragment to another dye or drug it should be possible to produce a compound having unique antitumor properties, or utility as probes of antitumor action. Recently, a compound having the covalent DNA-binding properties of an analog and the intercalating features of acridine orange has been synthesized. Upon binding to DNA, high concentrations of the dye increase the accessibility of bases in DNA to chemical reactions. The same synthetic scheme permits attachment of fluorescent rhodamine dyes to the "$PtCl_2$" moiety. Follow-up research relates to conversion of a modified bleomycin with a spermidine tail into a platinum derivative which could significantly affect the binding of cis-DDP to DNA *in vivo*.

These studies lead to better understanding of the mechanism of action of cis-DDP and contribute to development of more effective cancer therapeutic agents. (CA 34992, Lippard).

Forskolin as a Potential Antimetastatic Agent

Continued emphasis is placed on the discovery and development of new natural products for eventual clinical application. Studies have revealed that shortly after adhesion to blood vessel endothelium, metastatic cells become surrounded by thrombotic material, apparently induced by the transient tumor cells. The search for a compound which would act as a potent platelet aggregation inhibitor while causing minimal host toxicity has centered on forskolin, a diterpene isolated from the roots of the Indian plant, *Coleus forskohlii*. Forskolin induces significant reductions (over 70%) in numbers of pulmonary tumor foci. No overt toxicity was observed.

Forskolin is a powerful stimulator of platelet adenylate cyclase. It causes a several-fold increase in intracellular concentrations of c-AMP. It is likely that forskolin may act synergistically with these natural compounds *in vivo* to block tumor cell-platelet interactions. The low observed toxicity in mice and its effective inhibition of *in vitro* tumor induced aggregation of human platelets may make it a potential drug for treating rapidly metastasizing human tumors. (CA 07340, Parks)

Cell Kinetics-Directed Treatment Schedules

Solid tumors contain relatively low growth fractions. Therefore, agents which induce cell cycle phase synchrony or those that direct non-dividing cells into the cell cycle for therapeutic attack are valuable adjuncts in combination chemotherapy. Considerable research effort is directed at means of manipulating tumor cell cycle kinetics to achieve maximum cell kill by phase-specific agents.

Compound 1,2:5,6-di-anhydro-galactitol (GAL) 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 GAL treatment in CHO cells, bleomycin, an agent most effective in the G_2-M phases, was administered. At 30 hour post-GAL 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. They provide the basis for development of clinical protocols involving kinetics-based treatment schedules for more effective treatment. (CA 15397, Barranco).

Gene Amplification and Methotrexate Resistance

The role of gene amplification in the acquisition of cellular resistance to methotrexate (MTX) has been studied in the human, mouse, rat and hamster cell lines. In each case, the gene coding for dihydrofolate reductase (DHFR), the target enzyme for MTX, undergoes repeated amplification with a concurrent increase in gene product. Recombinant DNA studies and DNA sequencing in each of the cell lines shows remarkable similarity in nucleotide base composition. Recently, amplification of the DHFR gene has been demonstrated in blood cell samples drawn in vivo from a human patient with chronic myeloid leukemia. Post-treatment levels of DHFR, following administration of MTX, were two to threefold higher than pretreatment levels. Amplification occurred primarily if not exclusively, in neoplastic cells. Repeated samples drawn from the same patient were employed to avoid problems associated with the development of increasing resistance in cultured tumor lines. Although the mechanism of amplification is not known as yet, studies with mouse cell lines indicate that brief inhibition of DNA replication or damage to DNA itself, increases the occurrence of MTX resistance due to gene amplification. Development of multidrug protocols utilizing an inhibitory agent that does not damage DNA initially may help circumvent this problem. (CA 16318, Schimke)

Multidrug-Resistant Human Tumor Cells

Following the significant finding by Schimke that amplified genes in tumor cells which code for dihydrofolate reductase play an important role in methotrexate resistance, other researchers are examining the molecular basis for this action as it relates to acquired drug resistance. In particular, evidence is being sought that the mechanism may initiate pleiotropic or cross resistance to agents that share similar chemical structures.

The experimental plans include the isolation of the human homologue of the Chinese hamster gene associated with multidrug resistance and analysis of its amplification and expression in human tumors resistant to chemotherapy. Studies include correlation of expression of specific genes with the tumor drug resistance profile and the tumor type; isolation of full-length cDNA clones corresponding to the cloned genes and determination of their DNA sequence; analysis by gene transfer of the functions of the cloned genes in the development of multidrug resistance. These studies will elucidate mechanisms of pleiotropic drug resistance to provide information critical to successful clinical treatment of resistant leukemias in humans. (CA 40333, Roninson).

Areas for Future Emphasis

Tumor Heterogeneity

Research emphasis continues to be placed on studies designed to characterize genetic, biochemical and pharmacological differences among cell populations in a given tumor type. Other support is directed at understanding heterogeneous cell population response to single and multi-agent treatment, and to the mechanisms of development of cross-resistance to commonly used anticancer agents. The results provide a rationale for preparing drugs designed to exploit cell differences by novel or improved target specific delivery systems, and for the biochemical manipulation of mechanisms unique to tumor cells.

In Vitro Clinical Tumor Model Systems

This program strongly emphasizes research leading to the development of in vitro assays which will effectively predict patient tumor response before chemotherapy commences. In the coming year support will be directed primarily to rapid and inexpensive screening assays designed to predict and quantitate drug sensitive and drug resistant tumor responses. Two advantages of this strategy are foreseen: clinicians will be able to administer more efficacious disease specific drug regimens than at present, and continued dependence on more costly in vivo animal model systems will be diminished.

Counteractive Measures Against Clinical Toxicity

There is increasing awareness that the use of known and established clinical drugs can be manipulated to induce more effective and longer responses in cancer patients. Toxicity of drugs such as methotrexate and adriamycin in higher doses can be modified by manipulation of dosage regimens or by employment of rescue factors with ultimate protection to patients from severe toxic side effects. Use of high-dose therapy with appropriate counteractive rescue agents appears to be especially promising for patients with metastatic solid tumors.

Other Program Activities

This program provided support for the following conferences in FY 1985.

1) Workshop on Folyl and Antifolyl Polyglutamates, held in Airlie, Virginia, November 14-17, 1984, organized by Dr. I. David Goldman, Medical College of Virginia, Richmond, VA. 2) Cancer Drug Resistance Workshop, XIV International Chemotherapy Congress, held in Kyoto, Japan, June 23-28, 1985, organized by Dr. Thomas C. Hall, University of Hawaii, Honolulu, HI. 3) Gordon Conference on Polyamines and Cancer, held in Meriden, New Hampshire, July 1-5, 1985, organized by Dr. David R. Morris, University of Washington, Seattle, WA. 4) Gordon Research Conference on Chemotherapy of Cancer, held in New London, New Hampshire, July 22-26, 1985, organized by Dr. Franco Muggia, New York University Medical School, New York, NY.

During the last year, 887 research papers have been published with partial or total support provided by this program.

ANNUAL REPORT OF THE LABORATORY OF BIOLOGICAL CHEMISTRY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

The Laboratory of Biological Chemistry was established in 1985 to identify as targets for drug design, cellular reactions that are critical to the control of tumor cell proliferation or differentiation. Recent advances in cell biology are evaluated for possible targets. Agents are designed to interact with these targets and are evaluated for biochemical and antitumor effectiveness. An important aspect of this mission is to develop appropriate in vivo systems to evaluate the chemotherapeutic effectiveness of agents shown to be active in simpler in vitro model systems. Accordingly, the Laboratory is involved in identifying endogenous factors present in vivo that modify drug action and influence differential toxicity with the aim of manipulating these factors to enhance antitumor activity. To accomplish this mission, several existing research units were combined through an extensive reorganization of the DTP intramural research program to form the new Laboratory of Biological Chemistry. These units were the former Drug Metabolism Section, LCP, the Applied Pharmacology Section, LMCB, the Cellular Pharmacology Section, LMCB, and Dr. Breitman's research unit from LMCB. Ongoing research projects were re-evaluated and approximately 50% of the resources was applied to non-traditional targets for antitumor drug design and study. These non-traditional targets include early key biochemical events signaling cell proliferation or differentiation. The other 50% of Laboratory resources was applied to the study of either traditional targets or active compounds with traditional or unknown mechanisms of action.

Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or overexpression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. We have therefore initiated projects to develop new chemotherapeutic agents to block the action of growth factors. Non-traditional targets selected for drug design and study include: second messengers inositol triphosphate and diacylglycerol; myristoylation of cellular oncogene products; and protein kinase C.

Recent findings indicate that inositol-phosphates formed from phosphatidylinositides may be the second-messengers which mediate the action of many growth factors. In addition, the product of two distinct oncogenes (src and ros) phosphorylate phosphatidylinositol and increase the levels of phosphatidylinositol polyphosphates in the membranes of cells transformed by these oncogenes. The synthesis of phosphatidylinositol polyphosphates and their subsequent hydrolysis therefore provide attractive well-defined targets for the design of inhibitors for use in chemotherapy. A project was initiated that

has two related goals. First, to determine the relative importance of the enzymatic reactions involved in inositol phosphate production as a signal initiating cell replication. Secondly, to design, synthesize, and evaluate drugs to inhibit this process, specifically inhibitors of the reactions of phosphatidylinositol polyphosphate synthesis and of phospholipase C, the enzyme which produces the inositol phosphates. Test systems have been developed to identify drugs that inhibit phosphatidylinositol turnover or synthesis. Several compounds are currently being synthesized for evaluation in these systems. These include: 2-deoxy-myo-inositol and 5-deoxy-myo-inositol which lack critical hydroxyls; and phosphonate analogues of phosphatidylinositol as potential non-hydrolizable antimetabolites.

It may be possible to alter the activity of an oncogene product by interfering with its localization in the plasma membrane. The early events signaling cell proliferation occur in the plasma membrane of the cell, the location of most of the known cellular oncogene products. Myristoylation has been shown to be critical for the membrane localization and cellular transforming activity of p60src and has been implicated for other transforming proteins. Three mechanisms for blocking myristoylation-dependent transformation are under investigation: (1) direct chemical inhibition of transforming protein myristoylation; (2) inhibition of translocation of the myristoylated transforming protein to the plasma membrane; and (3) displacement of the myristoylated transforming protein away from the membrane, into the cytoplasm, and thus away from its substrate. Methods developed in the p60src system will be applied to malignant cells where membrane-associated oncogene products are suspected.

Inducers of protein kinase C activity such as diacylglycerols and phorbol esters, have shown the importance of this enzyme in the regulation of growth factor activity, lymphoblastogenesis, normal cell proliferation, cellular differentiation and oncogene expression. Studies in this Laboratory of protein kinase C activity during differentiation of human promyelocytic leukemia cell line HL-60 have indicated that not only is this enzyme activity increased in cells induced to differentiate along the myeloid or monocytic pathways, but that there is the rapid appearance of differentiation specific peptides which are phosphorylated in a calcium- and phospholipid-dependent manner. Natural metabolites that produce differentiating activity and activation of protein kinase C and phosphatidylinositol turnover are the diacylglycerols. Related dialkylglycerols and dialkylphospholipid analogues that are more metabolically stable are under investigation for their potential as selective anticancer drugs via their ability to induce differentiation of HL-60 cells. Currently 1-O-hexadecyl-2-O-methyl-3-phosphocholine and 1-O-hexadecyl-2-O-methyl-3-phosphate are being evaluated. Other analogues are being synthesized.

The availability of tissue culture cell lines has made it possible to study the regulation of proliferation and differentiation of specific hematopoietic cell types and the effects on these cells of known or suspected mediators and modulators. It was found previously in this Laboratory that retinoic acid (RA) is a potent inducer of terminal differentiation of the human promyelocytic cell line, HL-60, and the human monoblast- and monocyte-like cell lines, U-937 and THP-1. In addition RA was found to induce differentiation of fresh cells in primary culture of patients with acute promyelocytic leukemia. While retinoic acid alone is capable of inducing terminal differentiation, combinations of a physiological concentration of RA (10 nM) and either cAMP inducing agents (e.g.,

cAMP, prostaglandin E, or cholera toxin) or the conditioned medium from either activated T-cells or human leukemic T-cell lines synergistically induced differentiation of HL-60. An activity called "differentiation inducing activity" or DIA has been purified from these conditioned media and attempts are now being made to produce monoclonal antibodies against this protein. Studies are now in progress to apply the results obtained in vitro with HL-60 to a transplantable HL-60 tumor carried in athymic nude mice.

The technique of photoaffinity labeling is being used to identify intracellular drug binding proteins associated with the cytotoxicity or resistance of antitumor agents. A radioactive photoactive analogue of vinblastine was prepared and found to interact with alkaloid binding proteins that have not been described previously. The relationship of drug-binding proteins to cellular drug-sensitivity and drug-resistance is being investigated. In a similar study, a radioactive photoactive analogue of daunorubicin was found to bind to specific proteins in a heart homogenate. Identification of these intracellular drug-binding proteins could lead to a better understanding of the cytotoxic effects of these agents.

The methods developed to study the non-traditional targets described above are also being used to study the biochemical effects of new or established antitumor agents. Investigators in this Laboratory have extensive experience in nucleotide metabolism. Cyclopentenyl cytidine (cCyd) was shown to have in vivo antitumor activity against L1210 and P388 leukemias. cCyd produced a rapid decrease in CTP synthesis in vitro and in vivo, concomitant with an inhibition of rRNA transcription, and to a lesser extent, tRNA, without changes in the processing of nucleolar RNA. In HL-60 cells, inhibition of CTP synthetase leads to rapid inhibition of DNA and c-myc mRNA synthesis which precedes the appearance of differentiated cells. The cyclopentenyl adenosine analogue, neplanocin A, was found to be an effective inhibitor of RNA and DNA methylation resulting from its conversion to an analogue of AdoMet, and that these effects appear to be responsible for reduced c-myc RNA expression and the induction of myeloid differentiation in this cell line. The cyclopentenyl analogues of other nucleosides (including modified nucleosides such as 5-fluorouridine) will be studied in these systems.

A project involving traditional targets for drug design and study is a continuing project to determine the relative dependency of host and tumorous tissues on de novo vs salvage pathways for the synthesis of pyrimidine and purine nucleotides in vivo. This project is divided into the following specific aims: to determine the physiologic importance of circulating pyrimidines and purines and their role in modulating the antitumor activity of antipyrimidine and antipurine chemotherapeutic agents; to study the liver as a modulator of circulating nucleosides and as a possible target for chemical manipulation; to develop agents to interfere with nucleoside salvage to be used in combination with inhibitors of de novo synthesis; to develop methodology for monitoring and quantitating the flux through the de novo pathways of host and tumorous tissues in vivo. Studies from our Laboratory demonstrated that low levels of pyrimidines and purines, within the physiological range, are capable of reversing the toxicity of inhibitors of purine and pyrimidine de novo synthesis. Also, at these low concentrations, cultured cells turn off their de novo pathways and use exclusively their salvage mechanisms. Thus, circulating nucleosides may be an important factor in antipyrimidine and antipurine chemotherapy.

To evaluate to what extent tumors in vivo depend on their salvage pathways to fulfil their nucleotide requirements, we have adapted the tumor model system developed by Gullino and coworkers, where a tumor (in our case Walker 256) is implanted into the ovarian fat pad of female Sprague-Dawley rats and isolated in a parafilm pouch in the subcutaneous space. This system will permit direct sampling of the interstitial fluid and a measurement of the blood flow and trans-tumor differential of nucleoside concentrations. With this information it will be possible to calculate for the first time the actual net hourly contribution of circulating nucleosides and bases to tumor nucleotide pools.

Research from this laboratory has shown that the isolated rat liver can act as a donor organ of nucleosides that can be salvaged in vivo by other organs or tumors. Manipulation of this donor organ to inhibit or increase biosynthesis of salvageable nucleosides could be useful in cancer chemotherapy. A recently completed study from our laboratory indicated that the hepatic production of pyrimidines utilizes intermediates of the urea cycle. This study utilized ^{14}C-bicarbonate. We are currently using $^{15}NH_4Cl$ and ^{15}N-glutamine to determine directly the contribution of CPS-I and CPS-II to the N_1 and N_3 positions of the uracil nucleus.

The data so far discussed in this section indicate that inhibition of nucleoside salvage could enhance the effectiveness of inhibitors of de novo nucleotide synthesis. An in vivo model system developed to study the metabolism of pyrimidine nucleosides present in the plasma of the mouse is currently being used to assess the effects on salvage, in vivo, of agents designed to block pyrimidine salvage. 3-Deazauridine (500 mg/kg) produced a marked (>70%) inhibition of uracil nucleotide formation from uridine in liver, kidney, and L1210 tumor cells. Dipyridamole (100 mg/kg) did not significantly reduce salvage of uridine by liver or kidney and produced only small, transient reductions in salvage by L1210 tumors. Dipyridamole pretreatment did not alter the rate of clearance of uridine from the plasma. We examined a series of 29 analogs of uridine, most with modifications at the 5'-position, as inhibitors of uridine kinase in vitro and of uridine salvage by intact L1210 cells. Cyclopentenyluracil is promising as an inhibitor of uridine salvage since it selectively blocks the salvage of uridine by cells, is resistant to phosphorolysis, and is not cytotoxic at biochemically active concentrations. Further development and assessment of the in vivo effectiveness of this analogue is underway.

Uridine Phosphorylase is under evaluation as a chemotherapeutic agent. Twenty-five units/mouse of uridine phosphorylase, purified by affinity chromatography, produced marked (>90%) inhibition of uracil nucleotide formation by L1210 tumor cells, with a recovery to 40% of control seen 12 h after injection of enzyme. Uridine phosphorylase alone produced a modest (20%) decrease in uracil nucleotides of L1210 ascites cells, but greatly depleted (>95%) pools in combination with PALA, an inhibitor of pyrimidine synthesis de novo. Studies in progress will establish the therapeutic effect of combinations of PALA and uridine phosphorylase.

We have recently developed a method, which is the only method available, to monitor the flux through the de novo pyrimidine pathway in vivo. $^{13}CO_2$ is administered to mice and the incorporation of ^{13}C into pyrimidine nucleotides is quantitated by GC/MS. Using this method we studied the effect of PALA, a

potent inhibitor of de novo pyrimidine synthesis, on pathway flux in tumors sensitive and resistant to PALA. During the past year, this method was used to study the effect of acivicin, a glutamine analogue undergoing clinical evaluation, on de novo pyrimidine synthesis in L1210 (sensitive to acivicin) and B16 melanoma (resistant to acivicin) in vivo. GC/MS techniques were developed to quantify isotopic abundances in cytosine, cytidine, adenine, adenosine, quanine, guanosine, inosine, and hypoxanthine. These techniques will allow us to quantitate the flux through the de novo purine pathway in vivo simultaneous with measurements of the de novo pyrimidine pathway. We developed a method to quantify de novo pyrimidine biosynthesis based on the isotopic enrichment of N1 and N3 atoms of the uracil nucleus when hepatocytes are exposed to ^{15}N-NH_4Cl or ^{15}N-glutamine. From these values we are able to calculate, utilizing probability statistics, the number of uracil molecules produced by the de novo pathway that are labeled and those that are not labeled. By determining the enrichment of specific atoms of the product (uridine) formed in a pathway we know the isotopic enrichment of the immediate precursor pool for that atom. Comparison of this value with the precursor enrichment value obtained for the cell homogenate will indicate if compartmentation of precursors is occurring. We have developed a general mathematical framework using linear algebra that is implemented in a computer program that will calculate the results of isotope dilution experiments for an arbitrary number of components. The generality of the framework has allowed us to develop a detailed mathematical treatment of biosynthetic studies which measure incorporation of a stable labeled precursor into a metabolic product.

The experience of investigators of this Laboratory in the pharmacology of antitumor agents was applied to several active antitumor agents under development by DTP for clinical trial. The time course and dose response curve for inhibition of DNA synthesis in mouse spleen and jejunum was determined for aphidicolin glycinate, a water soluble ester of aphidicolin currently undergoing preclinical toxicological evaluation. Studies of the biochemical and pharmacologic effects of tiazofurin were continued. The combination of tiazofurin and 5-FU was synergistic in toxicity to L1210 cells; tiazofurin enhanced the toxicity of 5-FU in non-tumored mice. These effects may be related to a tiazofurin-induced increase in PRPP pools. The perfluorocarbon emulsion fluosol-DA was evaluated for its ability to increase the perfusion of solid tumors in vivo. Blood flow in a s.c. implanted solid Walker 256 tumor and in normal rat tissues was determined by 14C-iodoantipyrine and autoradiographic techniques. Fluosol-DA did not increase flow to the whole tumor and the pattern of regional flow within the tumor was not substantially altered; whereas cerebral flow was increased two-fold. A gas chromatographic method was developed using nitrogen-phosphorous detectors to quantify thiotepa and its metabolite, TEPA, in extracts of blood and CSF. This technique was used to study the pharmacokinetics of these two compounds in monkey and man following i.v. and i.t. administration. A method was developed to quantify intracellular thioinosine monophosphate and thioguanosine monophosphate to be used in a clinical study of 6-mercaptopurine. A GC/MS technique was developed for dihydrolemperone, an agent scheduled for clinical evaluation against lung cancer.

The preceding summary outlines the objectives of the Laboratory of Biological Chemistry and describes some of the research carried out within the Laboratory during the past year. The individual Project Reports, which follow, describe this research in greater detail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06162-01 LBC

PERIOD COVERED: October 1, 1984 to September 30, 1985

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	P.A. Monks	Visiting Associate	LBC, NCI		
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	R.L. Felsted	Research Chemist	LBC, NCI		
	C.J. Glover	Microbiologist	LBC, NCI		
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COOPERATING UNITS *(if any)*

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Pediatric Oncology Branch, NCI (S. Zimm and D. Poplack)
Clinical Pharmacology Branch, NCI (J. Collins)

LAB/BRANCH: Laboratory of Biological Chemistry

SECTION:

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	1.5	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.)*

The time course and dose response curve for inhibition of DNA synthesis in mouse spleen and jejunum was determined for aphidicolin glycinate, a water soluble ester of aphidicolin currently undergoing preclinical toxicological evaluation. Studies of the biochemical and pharmacologic effects of tiazofurin were continued. The combination of tiazofurin and 5-FU was synergistic in toxicity to L1210 cells.; tiazofurin enhanced the toxicity of 5-FU in non-tumored mice. These effects may be related to a tiazofurin-induced increase in PRPP pools. Cyclopentenyl cytosine was found to have reproducible antitumor activity against L1210 and P388 leukemia in the mouse. A sharp drop in CTP pools of L1210 cells in vivo was observed after treatment, indicating an effect on CTP synthetase in vivo. The perfluorocarbon emulsion fluosol-DA was evaluated for its ability to increase the perfusion of solid tumors in vivo. Blood flow in a s.c. implanted solid Walker 256 tumor and in normal rat tissues was determined by 14C-iodoantipyrine and autoradiographic techniques. Fluosol-DA did not increase flow to the whole tumor and the pattern of regional flow within the tumor was not substantially altered; whereas cerebral flow was increased two-fold. A gas chromatographic method was developed using nitrogen-phosphorous detectors to quantify thiotepa and its metabolite, TEPA, in extracts of blood and CSF. This technique was used to study the pharmacokinetics of these two compounds in monkey and man following i.v. and i.t. administration. A method was developed to quantify intracellular thioinosine monophosphate and thioguanosine monophosphate to be used in a clinical study of 6-mercaptopurine. A radioactive photoactive anthracycline analog was used to photoaffinity label anthracycline binding proteins in P388 cells sensitive and resistant to anthracyclines, and in rat heart homogenates. Specific polypeptides were isolated which may function as mediators of anthracycline activity.

PHS 6040 (Rev. 1/84)

GPO 914-918

Aphidicolin glycinate: Antitumor activity and biochemical effects in vivo. Aphidicolin, a specific inhibitor of DNA polymerase is active against experimental murine tumor models. Its poor solubility has led us to investigate aphidicolin glycinate (AG, NSC-303812), a water-soluble ester currently undergoing preclinical toxicological evaluation. AG has demonstrated good activity against the i.p. implanted B16 melanoma producing maximum increased life spans of 75% following i.p. administration q3h t.i.d. on days 1-9. Treatment schedules involving both single injections per day on days 1-9 and multiple injections per day on days 1,5, and 9 were less effective. Similarly, greater activity was observed against the i.p. M5076 sarcoma when 3 daily injections were given on days 1-9 (57% ILS) than with a single injection on either days 1-9 (36% ILS) or on days 1,5, and 9 (inactive). On the assumption that DNA polymerase inhibition is the basis for this activity, inhibition of DNA synthesis in CDF1 mice was evaluated by measurements of incorporation of ^{3}H-thymidine (20 μCi iv) into DNA of spleen and jejunum. At 2h after administration of AG, inhibition was dose dependent (ID_{50} 60 mg/kg in both tissues) and was >99% at 300 mg/kg. The inhibition was rapid in onset; AG 100 mg/kg i.p. produced maximal (>98%) inhibition in both tissues at 30 min. Recovery occurred in intestine within 16h; in spleen recovery was delayed to 24h, and was followed by a rebound incorporation at 48h (203%). We conclude that AG produces marked inhibition of DNA synthesis at doses below the LD_{10} in mice. Similar experiments with sensitive and resistant tumors may reveal selectivity in the inhibition of DNA synthesis, and guide optimal scheduling.

Tiazofurin: Biochemical and pharmacologic effects. We have shown that Tiazofurin (TR) enters human erythrocytes by utilizing their facilitated nucleoside transport system. TR competes with endogenous nucleosides for this transport mechanism, thereby reducing nucleoside uptake into the cells. The Km and Vmax (± SE) for radio-labeled TR transport into erythrocytes are 170 ± 26 μM and 55 ± 13 nmol/h/10^{6} cells, respectively, which is similar to the kinetic constants measured for uridine transport into erythrocytes (Km = 168 ± 37 μM and Vmax = 61 ± 16 nmol/h/10^{6} cells). The Ki (± SE) of TR for uridine transport is 178 ± 11 μM and for thymidine transport is 102 ± 59 μM. The clinical significance of TR sharing the same transport protein as endogenous nucleosides is unclear. TR could potentially reduce the entry of circulating nucleosides into cells and might thereby alter the effects of antipyrimidine antitumor agents, or it may directly alter the cellular transport of antitumor agents which utilize the nucleoside transport system.

Preliminary results from cell survival studies (using a cell cloning assay) show that a combination of 100 μM TR and 100 μM 5FU increases the killing of cultured L1210 cells by 1 log as compared to each of these agents alone. Furthermore, there is a significantly greater cell killing effect when these 2 drugs are combined if TR and 5FU are administered simultaneously or when TR incubation precedes TR incubation. This indicates that TR may be enhancing the cytotoxicity of 5FU. To test this hypothesis we studied biochemical changes in L1210 cells in vitro following TR treatment and showed that incubation of L1210 cells for 4 h with 0.5 or 2 mM TR resulted in a 7-9 fold increase in their intracellular concentration of PRPP. In addition, further incubation of these cells with ^{14}C-5FU for 1 h resulted in a 4-7 fold increase in the formation of phosphorylated anabolites of 5FU. These results indicate that the increased toxicity resulting from the combination of TR and 5FU may be

due to increased PRPP concentrations that enhance the anabolism of 5FU to toxic species.

When non-toxic doses (250-500 μM) of TR precede administration of 5FU to CDF_1 mice, there is an increase in host toxicity in response to this combination as compared to the administration of 5FU as a single agent. Administration of 200 mg/kg 5FU (i.p.) alone, resulted in a 7% mortality rate, which was increased to 27 and 63% with prior administration of 250 or 500 mg/kg TR. When mice bearing L1210 tumors were pretreated with 250 mg/kg TR, there was an improvement in the antitumor effect of a low dose of 5FU (50 mg/kg) (42% ILS 5FU alone to 85% ILS 5FU/TR combination), but this combination was not superior to the antitumor properties of an optimal dose (100 mg/kg) of 5FU alone (85% ILS). Thus, it appears that the dose response curve for 5FU toxicity is shifted to lower concentrations by TR.

Pharmacology of cyclopentenyl cytosine. Cyclopentenyl cytosine is currently under evaluation as an antitumor agent: reproducible activity has been seen against both L1210 and P388 leukemia in the mouse. In L1210 a >100% ILS was achieved when mice were treated with 1 mg/kg qdx9. Biochemical studies in vivo and in cell cultures suggest inhibition of cytidine nucleotide formation as the mechanism of action. In vivo treatment of mice bearing L1210 leukemia produces a sharp drop in CTP pools within 4h. In L1210 cell cultures, cyclopentenyl cytosine inhibits cell replication at concentrations as low as 100 nM, but provision of 20 μM cytidine permits cell growth at normal rates even in the presence of 1 μM cyclopentenyl cytosine.

Attempts to increase blood flow in the rat solid Walker 256 tumor by the use of perfluorochemical emulsion fluosol-DA (F-DA). Solid tumors may be refractory to antineoplastic agents because of poorly perfused regions within the tumor. The small particle size and decreased viscosity of the F-DA emulsion as compared to blood may allow for increased perfusion throughout the tumor. We have examined the effect of F-DA on blood flow in male Wistar rats bearing a Day-7 solid Walker 256 tumor implanted s.c. in the neck. F-DA-treated rats maintained under 100% O_2 underwent an isovolumic F-DA-blood exchange which reduced their hematocrits to less than 3%. Blood flow in tumor and normal tissues was determined using ^{14}C-iodoantipyrine and autoradiographic techniques. The results indicate that F-DA blood exchange did not increase flow to the whole tumor. Similarly the pattern of regional flow within the tumor was not substantially altered. In contrast, flow to cerebral tissue was increased two-fold. The findings do not support the use of F-DA to enhance blood flow to the s.c. implanted solid Walker 256 tumor.

Anthracycline photoaffinity labeling of an 18 Kdalt protein in P388 sensitive and resistant cell lines. N-(p-Azidobenzoyl)-daunorubicin, ([^3H]NABD), a radioactive photoactive anthracycline analog was used for photoaffinity labeling of anthracycline binding polypeptides (ABPs) in P388 murine leukemic cells. Anthracycline sensitive (P388/S) and resistant (P388/DRN) cell homogenates were mixed with 50 nM [^3H]NABD, exposed to UV-light, and analyzed by SDS-PAGE for ^3H incorporation. Fluorography showed incorporation of radioactivity into a M_r 18 kdalt component independent of prominently stained polypeptides. Radioactive incorporation into this component was 3966 ± 355 dpm and 6487 ± 533 dpm per 50 μg cellular protein for P388/S and P388/DRN cells, respectively ($p<.005$). Photolabeling of P388/S subcellular fractions showed predominant

mitochondrial localization of the M_r 18 kdalt radiolabel. This was confirmed by a relative quantitative distribution of radiolabel corresponding to that of the mitochondrial marker, cytochrome oxidase. ABP photolabeling was unaffected by DNAse or RNAse, and radioactivity was not extracted into chloroform:methanol (2:1). The protein composition of the photolabeled constituent was confirmed by the reduction of radioactivity to ambient background following treatment with proteinase K. The quantitative photolabeling of M_r 18 kdalt was maximum after 10 min of UV-light exposure, and was linear with respect to protein. Saturation of photolabeling was observed for higher concentrations (>100 nM) of [^{3}H]NABD. The possible role of this polypeptide as a mediator of anthracycline activity remains to be determined.

Rat heart anthracycline binding polypeptides (ABPS) identified by photo-affinity labeling. The same technique described above was used to photo-affinity label ABPs in rat heart homogenate. The most prominently labeled species were M_r 18.3 and 31.2 Kd polypeptides located in the 100,000 x g sedimented fraction. M_r 18.3 exhibited a saturable photolabeling of 2.42 ± 0.47 pmoles/mg homogenate protein above 0.15 μM ^{3}HNAB-daunorubicin while M_r 31.2 exhibited an apparent linear increase in photolabeling up to 0.62 μM ^{3}H-NAB-daunorubicin. A 16-fold molar excess of NAB-daunorubicin over 0.16 μM ^{3}H-NAB-daunorubicin reduced the photolabeling of M_r 18.3 by 90% confirming a high degree of analog labeling specificity. M_r 31.2 was reduced 25% suggesting both specific and nonspecific labeling. M_r 18.3 and 31.2 were not labeled by photoactive model compounds lacking the amino sugar and/or the anthracyclinone ring. Also, photolabeling was inhibited by the addition of daunorubicin and N-substituted anthracycline analogs.

Clinical pharmacologic studies with 6-mercaptopurine. A method was developed to quantify intracellular thioinosine monophosphate (TIMP) and thioguanosine monophosphate (TGMP) in patients' leukemic cells which were treated with 6-mercaptopurine (6MP). The feasability of the method was demonstrated by quantifying TIMP and TGMP in leukemic cells obtained 24 h and 48 h after start of infusion with 6MP. These studies will be used to correlate TIMP and TGMP intracellular levels with plasma levels of 6MP and the patient's response.

Blood-CSF studies with thiotepa. The intent of this study was to evaluate the therapeutic advantage of intrathecal administration of thiotepa using a monkey model. A gas chromatographic method was developed using nitrogen-phosphorous detectors to quantify thiotepa and its metabolite, TEPA, in extracts of blood and CSF. The results of these studies showed that after iv administration of thiotepa, the drug was rapidly equilibrated with CSF (<10 min.), and that thiotepa was rapidly cleared from CSF and plasma (t 1/2 of approx. 20-30 min). However, after iv administration of thiotepa, considerable TEPA was formed and found to have a much longer t 1/2 (approx. 5 h). A comparison of total tissue exposure (AUC) of thiotepa and TEPA to the CSF after iv and i.t. administration of Thiotepa indicated a 20:1 therapeutic advantage when the drug was given iv (assuming that the potency of thiotepa and TEPA are equivalent). Subsequent data obtained from a patient who received an i.t. dose and another patient who received an iv dose of Thiotepa indicated species differences in man and monkey in that the amount of TEPA formed in man was considerably less than that observed in the monkey. These results suggest no therapeutic advantage of iv administration of Thiotepa and demonstrate that the monkey model is a poor predictor for man in this instance.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06163-01 LBC

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
Pharmacologic Aspects of Nucleotide Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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	N. Malinowski	Chemist	LBC, NCI		
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LAB/BRANCH
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SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.5	2	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.)*

The isolated perfused rat liver exports the purines adenine and hypoxanthine at a rate such that 0.5-1.0 μM of each compound is maintained in the perfusate. It was found that this concentration of either purine completely reversed the growth inhibition of L1210 and HL-60 cells caused by DON, an inhibitor of de novo purine biosynthesis. Also, 1 μM adenine (in the absence of DON) completely inhibited de novo purine synthesis. Thus, physiologic concentrations of these purines can support cell growth in the absence of de novo synthesis. An in vivo tumor model was established to study the actual utilization of circulating purines and pyrimidines by a solid tumor. Studies with 15NH4CL and 15N-glutamine demonstrated extensive involvement of the urea cycle in the formation of pyrimidines, de novo, in isolated rat hepatocytes. 3-Deazauridine and dipyridamole were evaluated as inhibitors of uridine salvage in vivo. 3-Deazauridine produced a marked, but short-lived, inhibition of uridine salvage in liver, kidney, and L1210 tumor cells. Dipyridamole did not significantly reduce salvage. A series of 29 analogues of uridine was examined as inhibitors of uridine kinase in vitro and of uridine salvage by intact L1210 cells. Of these compounds, cyclopentenyl uracil is most promising since it selectively blocks the salvage of uridine by L1210 cells, is resistant to phosphorolysis, and is non-cytotoxic at biochemically active concentrations. Uridine phosphorylase was purified to homogeneity and tested in vivo for its ability to reduce the concentration of circulating uridine that would be available for salvage. Uridine phosphorylase alone produced a modest (20%) decrease in uracil nucleotides of L1210 ascites cells, but greatly depleted (>95%) pools in combination with PALA, an inhibitor of pyrimidine synthesis de novo. A series of uridine analogues modified in the 5'-position of the ribosyl moiety was evaluated as inhibitors and substrates of uridine phosphorylase. Replacement or esterification of the 5'-hydroxyl group led to nearly complete loss of susceptibility to phosphorolysis.

GPO 914-918

Objective:

The overall objective of this project is to determine the relative dependency of host and tumorous tissues on de novo vs salvage pathways for the synthesis of pyrimidine and purine nucleotides in vivo. The de novo biosynthetic pathways supply pyrimidines and purines for nucleic acid synthesis and are therefore considered to be important pathways for cell proliferation. Therefore, there has been a considerable effort throughout the past several decades to develop specific inhibitors of enzymes of these pathways. Although a number of potent inhibitors (e.g. PALA, pyrazofurin, 6-azauridine) exhibit excellent in vitro activity against isolated enzymes and cultured cells and in vivo activity against certain murine tumors, only marginal clinical success has been achieved with these agents. It would appear that factors other than potency of inhibition are important because very potent enzyme inhibitors (PALA, pyrazofurin) are only marginally effective. Accordingly, lack of clinical success with the pathway inhibitors developed to date might be an indication that the importance of the de novo pathway to cell survival in vivo might be over-estimated. If so, then the development of additional inhibitors of the de novo pathway would be a futile effort unless there is a coordinate development of agents that either interfere with the salvage pathway or with the synthesis and export of preformed nucleosides by donor organ(s).

This Project is divided into the following specific aims: to determine the physiologic importance of circulating pyrimidines and purines and their role in modulating the antitumor activity of antipyrimidine and antipurine chemotherapeutic agents; to study the liver as a modulator of circulating nucleosides and as a possible target for chemical manipulation; to develop agents to interfere with nucleoside salvage to be used in combination with inhibitors of de novo synthesis; to develop methodology for monitoring and quantitating the flux through the de novo pathways of host and tumorous tissues in vivo.

Major Findings:

Physiologic and pharmacologic importance of circulating nucleosides. Previous studies from our Laboratory (Karle, Anderson, and Cysyk, J. Biol. Chem. 259: 67-72, 1984) demonstrated that uridine, at concentrations found in plasma, reversed the growth inhibition by PALA of cultured L1210 cells. Also, in the presence of plasma concentrations of uridine, cultured L1210 cells turned off de novo pyrimidine synthesis and utilized their salvage pathway nearly exclusively. Thus, circulating uridine may be an important factor in antipyrimidine chemotherapy. Purine nucleosides and bases were studied in a similar manner. It was determined that the isolated perfused rat liver exports the purines adenine and hypoxanthine at a rate to maintain the perfusate at 0.5-1.0 μM in each of these compounds. HL-60 cells were treated with 3 μM DON and L1210 cells were treated with 0.3 μM DON. Concurrently, the cells were treated with 0.5 to 10 μM adenine or hypoxanthine. Treatment with DON, only, resulted in inhibition of cell growth to 30% of control growth. Co-incubation with low levels of either adenine or hypoxanthine reversed this toxicity such that the cells grew 80-95% of control growth. Thus, low levels of purines, within the physiological range, are capable of reversing the toxicity of an inhibitor of purine de novo synthesis. It was found that adenine concentrations as low as 1.0 μM completely inhibit purine de novo synthesis. At this concentration, PRPP levels are unaffected as is uridine

synthesis. Thus, if maintained at a constant concentration, 1 μM adenine is able to provide sufficient purines to maintain the purine nucleotide pool via the salvage pathway alone. In addition, the regulation of the de novo synthetic pathway is accomplished by a means other than lowering of the PRPP pool, since the PRPP concentration was unaffected by an adenine concentration capable of completely inhibiting purine de novo synthesis.

Development of an in vivo tumor model to study the utilization of circulating preformed purines and pyrimidines. To evaluate to what extent tumors in vivo depend on their salvage pathways to fulfil their nucleotide requirements, we have adapted the tumor model system developed by Gullino and coworkers, where a tumor (in our case Walker 256) is implanted into the ovarian fat pad of female Sprague-Dawley rats and isolated in a parafilm pouch in the subcutaneous space. This "tissue-isolated" tumor is connected to the host by a single artery and vein from which blood can be withdrawn. Also, a chamber can be inserted into the growing neoplastic mass from which to sample the interstitial fluid. Guillino and coworkers used this system to analyze for amino acids, proteins, and a variety of small molecules (other than nucleosides). This system permits direct measurement of the blood flow and trans-tumor differential of nucleoside concentration. With this information it will be possible to calculate for the first time the actual net hourly contribution of circulating nucleosides and bases to tumor nucleotide pools.

Hepatic regulation of circulating nucleosides. Research from this laboratory (Monks and Cysyk, Am. J. Physiol. 242, 465, 1982) has shown that the isolated rat liver can act as a donor organ of the nucleoside uridine which could be salvaged in vivo by other organs or tumors. Manipulation of this donor organ to inhibit or increase biosynthesis of salvageable uridine could be useful in cancer chemotherapy. A recently completed study from our laboratory (Monks, Chisena, and Cysyk, Arch. Biochem. Biophys. 236: 1, 1985) indicated that the hepatic production of pyrimidines utilizes intermediates of the urea cycle. This study utilized ^{14}C-bicarbonate. We are currently using $^{15}NH_4Cl$ and ^{15}N-glutamine to determine directly the contribution of CPS-I and CPS-II to the N_1 and N_3 positions of the uracil nucleus. The extent of incorporation is determined by GC/MS using our recently developed method (Strong et al. J. Biol. Chem. 260: p. 4276, 1985).

Development of inhibitors of nucleoside salvage. The results discussed in the earlier sections of this project indicate that inhibition of nucleoside salvage could enhance the effectiveness of inhibitors of de novo nucleotide synthesis. An in vivo model system (Moyer, Malinowski, and Ayers, J. Biol. Chem. 260: 2812-2818, 1985) developed to study the metabolism of pyrimidine nucleosides present in the plasma of the mouse is currently being used to assess the effects on salvage, in vivo, of agents designed to block pyrimidine salvage. A series of studies were performed to evaluate the effects of 3-deazauridine and the nucleoside transport inhibitor dipyridamole (Persantin) on uridine salvage in vivo. 3-Deazauridine (500 mg/kg) produces a marked (>70%) inhibition of uracil nucleotide formation from uridine in liver, kidney, and L1210 tumor cells. The inhibition was only short-lived however, even at this high dose. Dipyridamole (100 mg/kg) did not significantly reduce salvage of uridine by liver or kidney and produced only small, transient reductions in salvage by L1210 tumors. Dipyridamole pretreatment did not alter the rate of clearance of uridine from the plasma.

We examined a series of 29 analogs of uridine, most with modifications at the 5'-position, as inhibitors of uridine kinase in vitro and of uridine salvage by intact L1210 cells. Substitution at the 5'-position resulted in decreased efficacy as inhibitors of uridine kinase, particularly if the substituent was large. None of the analogs with 5'-position modifications effectively inhibited salvage of uridine by intact L1210 cells. A series of four carbocyclic pyrimidine nucleoside analogs were all effective competitive inhibitors of uridine kinase and of uridine salvage by intact L1210 cells. Cyclopentenyluracil is promising as an inhibitor of uridine salvage since it selectively blocks the salvage of uridine by cells, is resistant to phosphorolysis, and is not cytotoxic at biochemically active concentrations. Although not a particularly potent inhibitor of uridine kinase it may nonetheless be effective in vivo, because physiological levels of uridine phosphorylase should prevent accumulation of uridine behind the enzymic block. Further development and assessment of the in vivo effectiveness of these analogues is underway.

Evaluation of Uridine Phosphorylase as a chemotherapeutic agent. Results published this year (Moyer and Henderson, Biochem. Pharm. 34: 101-105, 1985) indicated that administration of uridine phosphorylase could reduce the utilization of endogenous uridine by conversion to uracil which is very poorly salvaged. We have subsequently found that 25 units/mouse of uridine phosphorylase, purified by affinity chromatography, produced marked (>90%) inhibition of uracil nucleotide formation by L1210 tumor cells, with a recovery to 40% of control seen 12 h after injection of enzyme. Uridine phosphorylase alone produced a modest (20%) decrease in uracil nucleotides of L1210 ascites cells, but greatly depleted (>95%) pools in combination with PALA, an inhibitor of pyrimidine synthesis de novo. Studies in progress will establish the therapeutic effect of combinations of PALA and uridine phosphorylase, but initial results indicate that the combination is more toxic than PALA alone, and this increase in toxicity may preclude successful use of this drug combination.

Development of inhibitors of nucleoside catabolism. The enzyme uridine phosphorylase (EC 2.4.2.3) catalyzes the first step of uridine catabolism and also cleaves the chemotherapeutic agents 5-fluorouridine, 5-bromo-2'-deoxyuridine, and 5-iodo-2'-deoxyuridine. In vivo this enzyme is extremely active and produces a very rapid cleavage of uridine in rats or mice. Susceptibility to cleavage by this enzyme is therefore a major consideration for the design of pyrimidines as chemotherapeutic agents. We examined a series of uridine analogs modified in the 5'-position of the ribosyl moiety as inhibitors and substrates of uridine phosphorylase from E. Coli and from rat liver. Replacement or esterification of the 5'-hydroxyl group led to nearly complete loss of susceptibility to phosphorolysis by either enzyme. 5'-Chloro-deoxyuridine and 5'-benzoyl-uridine, although not substrates, were competitive inhibitors of uridine phosphorylase from E. Coli with K_i's of 370 μM and 49 μM, respectively. 5'-Amino-5'deoxyuridine activated uridine phosphorylase from E. Coli with both a decrease in Km for uridine and an increased V_{max} observed. These results are important to the design of uridine analogs and for the mechanism of uridine phosphorylase.

Publications:

1. Karle, J.M., Anderson, L.W. and Cysyk, R.L.: Effect of plasma concentration of uridine on pyrimidine biosynthesis in cultured L1210 cells. J. Biol. Chem.: 259, 67-72, 1984.

2. Karle, J.M. and Cysyk, R.L.: Regulation of pyrimidine biosynthesis in cultured L1210 cells by 3-deazauridine. Biochem. Pharmacol. 33: 3739-3742, 1984.

3. Karle, J.M., Monks, A., Wolfe, R.M. and Cysyk, R.L.: Effect of 2-β-D-Ribofuranosylthiazole-4-carboxamide on salvage of nucleosides by cultured L1210 cells. Cancer Lett. 24: 4276-4281, 1985.

4. Lim, M., Moyer, J.D., Cysyk, R.L. and Marquez, V.E.: Cyclopentenyl uridine and cytidine analogs as inhibitors of uridine-cytdine kinase. J. Med. Chem. 27: 1536-1538, 1984.

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6. Moyer, J.D. and Paterson, A.R.P. and Henderson, J.F.: Effect of a nucleoside transport inhibitor on the disposition of uridine in mice. Biochem. Pharmacol. 33: 2327-2328, 1984.

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8. Moyer, J.D., Malinowski, N., and Ayers, O.: Salvage of circulating pyrimidine nucleosides by tissues of the mouse. J. Biol. Chem. 260: 2812-2818, 1985.

9. Strong, J.M., Upton, D.K., Anderson, L.W., Monks, A., Chisena, C.A. and Cysyk, R.L.: A novel approach to the analysis of mass spectrally assayed stable isotope labeling experiments. J. Biol. Chem. 260: 4276-4281, 1985.

10. Zimm, S., Grygiel, J.J., Strong, J.M., Monks, T., Poplack, D.G.: Indentification of 6-mercaptopurine riboside in patients receiving 6-mercaptopurine as a prolonged intravenous infusion. Biochem. Pharmacol. 33: 4089-4092, 1984.

11. Karle, J.M., Cowan, K.H. and Cysyk, R.L.: Uracil nucleotide synthesis is a human breast cancer cell line (MC-7) and in two drug resistant sublines that contain increased levels of enzymes of the de novo pyrimidine pathway. J. Biol. Chem., 1985, in press.

12. Moyer, J.D. and Henderson, J.F.: Compartmentation of intracellular nucleotides in mammalian cells. Crit. Rev. Biochem., 1985, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06164-01 LBC

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Inhibitors of Phospholipid Metabolism as Potential Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Moyer	Staff Fellow	LBC, NCI
Other:	N. Dean	Visiting Fellow	LBC, NCI
	O. Ayers	Lab. Technician	LBC, NCI

COOPERATING UNITS *(if any)*

Medicinal Chemistry Section, LPET, DCT, NCI
Drug Synthesis and Chemistry Branch, DCT, DTP, NCI.

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	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.)*

Agents which block the formation of second messengers which mediate growth factor action may be of value in cancer chemotherapy. We have developed test systems to identify drugs which interfere with phosphatidylinositol formation or hydrolysis, because the formation of inositol phosphates has been implicated in the action of a number of growth factors including platelet derived growth factor and bombesin. A series of rationally-designed inhibitors of enzymes involved in phosphatidylinositol metabolism are currently being synthesized for evaluation.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives: Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or overexpression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. We have therefore initiated a project to develop new chemotherapeutic agents to block the action of growth factors.

Recent findings indicate that inositol-phosphates formed from phosphatidylinositides may be the second-messengers which mediate the action of many growth factors. In addition, the product of two distinct oncogenes (src and ros) phosphorylate phosphatidylinositol and increase the levels of phosphatidylinositol polyphosphates in the membranes of cells transformed by these oncogenes. The synthesis of phosphatidylinositol polyphosphates and their subsequent hydrolysis therefore provide attractive well-defined targets for the design of inhibitors for use in chemotherapy. This project has two related goals. First, to determine the relative importance of the enzymatic reactions involved in inositol phosphate production as a signal initiating cell replication. Secondly, to design, synthesize, and evaluate drugs to inhibit this process, specifically inhibitors of the reactions of phosphatidylinositol polyphosphate synthesis and of phospholipase C, the enzyme which produces the inositol phosphates.

Major Findings: A series of test systems have been developed to identify drugs that inhibit phosphatidylinositol turnover or synthesis. These test systems fall into three categories: Functional tests of mitogen action, biochemical assay of inositol phosphate production, and assays of the enzymes involved in phosphatidylinositol metabolism.

Functional Assays of Mitogen Action: Two assays have been developed based on the response of quiescent Swiss 3T3 cells to serum or purified factors such as phorbol esters or platelet derived growth factor (PDGF). The first is [^{3}H]-thymidine incorporation as a measure of DNA synthesis, the other is [^{3}H]uridine uptake. Uridine uptake is reproducibly enhanced 3 to 5-fold within 1 h of stimulation of 3T3 cells by serum, phorbol esters, or PDGF. This assay should identify agents which prevent the action of growth factors, and is rapid and simple enough to permit testing of about 20 compounds per day.

Biochemical Assays of Inositol Phosphate Production: We have developed an assay for measuring [^{3}H]-inositol phosphates based on separation with high performance liquid chromatography. Use of an automatic sample injector and a microprocessor-controlled fraction collector has permitted automation of this assay. Initial studies demonstrate a 3-5 fold increase in [^{3}H]-inositol phosphate concentrations in 3T3 cells prelabeled with [^{3}H]-inositol and stimulated with serum. This assay is being further characterized and should identify agents which block the formation of inositol phosphates in 3T3 cells.

A second assay employs GH_3 cells, a rat pituitary tumor cell line. These cells were found to produce particularly large amounts of inositol phosphates when stimulated by TRH in the presence of lithium. The large production of inositol

phosphates (50-fold controls) permits an estimate of inositol phosphate formation without chromatography and makes these cells an excellent test system. We anticipate about 30 compounds per day could be evaluated.

Phosphatidylinositol synthesis by intact GH_3 cells can also be measured in a short term assay of [^{3}H]-inositol incorporation. Incorporation is directly proportional to time for at least 4 h and >90% of the label present in the lipid fraction is phosphatidylinositol as determined by thin layer chromatography.

Enzyme Assays: A soluble preparation of phosphatidylinositol synthetase permits enzyme activity measurements and the identification of inhibitors and substrates for this enzyme. Initial results show that simple di-substituted cyclohexanes are not inhibitors nor is epi-quercitol (4-deoxy-myo-inositol). The enzyme is inactivated by α,β unsaturated cyclohexone. The Km for inositol was measured at 1.7 mM.

Proposed Course: Several compounds are currently being synthesized for evaluation in the assays described above. In particular, the 2-deoxy-myo-inositol and 5-deoxy-myo-inositol, which lack critical hydroxyls, will be evaluated. Mechanistic considerations suggest that these compounds should be unable to serve as a source of second messengers if they can be incorporated into phospholipids. Phosphonate analogs of phosphatidylinositol are potential non-hydrolizable analogs with possible inhibitory action. We will also attempt to alter cellular content of phosphatidylinositides to determine the relative importance of phosphatidylinositide availability versus phospholipase activation in the production of inositol phosphates after stimulation by growth factors. These studies may provide useful inhibitors of phospholipid metabolism and should further clarify the potential of phospholipid metabolism as a target for cancer chemotherapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06165-01 LBC

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Stable Isotope Studies of de Novo Pyrimidine and Purine Pathways

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Strong	Pharmacologist	LBC, NCI
Other:	L.W. Anderson	Chemist	LBC, NCI
	D.W. Zaharevitz	Staff Fellow	LBC, NCI
	R.L. Cysyk	Pharmacologist	LBC, NCI

COOPERATING UNITS *(if any)*

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Laboratory of Biological Chemistry

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1	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.)*

Existing stable isotope tracer methodology was used to monitor the flux through the de novo pyrimidine pathway in vivo. Recovery of pathway activity after acivicin treatment in acivicin-sensitive and acivicin-resistant tumor lines was determined. In conjunction with these studies a new tumor model using invaded spleens was evaluated. Stable isotope tracer methodology was developed for studying the de novo pyrimidine and purine pathways. Methodology was developed using GC/MS techniques in combination with HPLC separations for quantifying isotopic abundances in both purine and pyrimidine bases and nucleosides. In addition, a fully automated GC/MS technique was developed to simultaneously quantify the amount and isotopic ^{15}N enrichment in amino acids in sample extracts of various tissues. A computer model using linear algebra techniques was developed which allows us to interpret data obtained from multiple stable label experiments. The predictive nature of this technique will allow us to study the pharmacologic effects of compartmentation as it relates to antitumor activity of agents which act to inhibit pyrimidine and purine biosynthesis.

PHS 6040 (Rev. 1/84)

GPO 914-918

Radiolabeled tracer methodology has played a major role in the elucidation of metabolic pathways. Such information, coupled with specific activity determinations of enzymes in cell-free extracts, is a valuable indicator of the importance of a particular pathway in the intact cell. Studies designed to measure pathway activity need to be carried out in intact cells because of the improbability of duplicating, in vitro, the intracellular microenvironment in which the enzyme functions. Moreover, the concept of intracellular compartmentation of pathway activities has now replaced the simplistic view of the cell as a bag of uniformly dispersed enzymes acting on a homogeneous supply of metabolites. Intracellular and intercellular compartmentation of enzymes and small molecules necessitates experimentation with intact cells and animals, however at the same time, complicates the interpretation of results obtained using tracer methodology. The use of stable labeled compounds as tracers combined with mass spectrometric techniques in some cases are more advantageous for these types of studies. Precursors can be labeled with the appropriate stable isotope to essentially 100% enrichment without posing a biohazard. These high enrichments minimize the dilution effects which occur especially in vivo. Stable isotopes such as nitrogen and oxygen which have no radiolabel isotopes easily available can be used as tracers and most importantly, these techniques are easily adapted to studies where several atoms in the product molecule are labeled. The latter advantage will allow us to address some of the problems with tracer studies such as the fact that isotope enrichment determinations in pathway products following administration of labeled precursors represent an average enrichment value which includes (a) isotopically enriched product molecules that were formed from the labeled pathway under study and (b) non-isotopically enriched product molecules that were either present prior to the labeling period or were produced by an alternate pathway during the course of the labeling experiment. A method which would distinguish a-type molecules from b-type molecules would be a desirable technique to study pathway activity and regulation. Furthermore, such a method would be valuable in compartmentation studies since the enrichment of the newly synthesized product molecules would be a measure of the isotopic enrichment of the precursor molecules in the cellular compartment from which the product is derived.

Objective:

The objective of this project is two-fold: (1) to develop stable-label methodology to quantitate the flux through the de novo pyrimidine and purine pathways in vivo and in intact cells in vitro, and (2) to develop stable-label methodology and computer models to study compartmentation of reaction pathways in vivo and in intact cells in vitro.

Measurement of Flux through the de novo pyrimidine and purine pathways. We have recently developed a method (Strong et. al., Anal. Biochem. 132: 243-253, 1983), which is the only method available, to monitor the flux through the de novo pyrimidine pathway in vivo. $^{13}CO_2$ is administered to mice and the incorporation of ^{13}C into pyrimidine nucleotides is quantitated by GC/MS. Using this method we studied the effect of PALA, a potent inhibitor of de novo pyrimidine synthesis, on pathway flux in tumors sensitive and resistant to PALA (Monks et. al., J. Biol. Chem. 258, 13564-13569, 1983). During the past year, this method was used to study the effect of acivicin, a glutamine analogue undergoing clinical evaluation, on de novo pyrimidine synthesis in L1210 (sensitive to acivicin) and B16 melanoma (resistant to acivicin) in vivo.

In conjunction with these studies, a new tumor model (tumor invaded spleens contained in L1210 sc tumored mice), was investigated. Flux through the de novo pathway as measured by incorporation of $^{13}CO_2$ into the Σ uracil pools of both tumors and L1210-invaded spleens was maximally suppressed 1 h after acivicin treatment. The inhibitory effect of acivicin was reversed by 4 h in L1210 sc and spleens; however, the inhibitory action of acivicin persisted for 48 hrs in the B16 melanoma model. Investigations are continuing using stable isotope labeling experiments to determine the effect of acivicin on the conversion of UTP to CTP in vivo.

Gas chromatographic/mass spectrometric (gc/ms) techniques were developed to quantify isotopic abundances in cytosine, cytidine, adenine, adenosine, quaninine, guanosine, inosine, and hypoxanthine. These gc/ms techniques in combination with HPLC separations and enzyme and chemical degradations, will allow us to quantify the isotopic abundances in the purine and pyrimidine bases arising from base, nucleoside, and individual nucleotide pools contained in various tissues. Investigations are presently underway to establish the appropriate precursors, i.e., $^{15}NH_4$, ^{15}N-glutamine, ^{15}N-glycine, etc., which will produce sufficient labeling in the purine nucleotide pools; thus allowing quantification of flux through the de novo purine pathway in vivo simultaneous with measurements of the de novo pyrimidine pathway. Other drugs and combinations of drugs will be studied in this system. Also, the dilabel technique (described below) will be used to quantitate pathway flux.

Pharmacologic considerations of pathway compartmentation. Recently we reported a method to quantify de novo pyrimidine biosynthesis based on the isotopic enrichment of N1 and N3 atoms of the uracil nucleus when hepatocytes are exposed to ^{15}N-NH_4Cl or ^{15}N-glutamine, (Strong et. al., J. Biol. Chem. 260: 4276-4281, 1985). From these values we are able to calculate, utilizing probability statistics, the number of uracil molecules produced by the de novo pathway that are labeled and those that are not labeled. By determining the enrichment of specific atoms of the product (uridine) formed in a pathway we know the isotopic enrichment of the immediate precursor pool for that atom. Comparison of this value with the precursor enrichment value obtained for the cell homogenate will indicate if compartmentation of precursors is occurring. We have developed a general mathematical framework using linear algebra that is implemented in a computer program that will calculate the results of isotope dilution experiments for an arbitrary number of components. The generality of the framework has allowed us to develop a detailed mathematical treatment of biosynthetic studies which measure incorporation of a stable labeled precursor into a metabolic product.

In order to implement these studies, it was necessary to develop methods to quantify the isotopic enrichments in the amino acids which are intermediates in the pyrimidine de novo synthetic pathway in vivo, especially glutamine and aspartate. This past year we developed a fully automated system using a Hewlett Packard gc/ms equipped with an automatic sampler to quantify both the amount of amino acid in the sample and the isotopic abundance of stable labeled atoms in the amino acids simultaneously, for aspartate, glutamine, glutamate, and the non-amino acid urea in a single gs/ms run. Preliminary studies in tumored mice which were infused i.p. with $^{15}NH_4Cl$ indicate the usefulness of predicting compartmentation of reaction pathways in vivo. Firstly, analysis of the uracil nucleotide pool formed during the course of the experiment showed

different patterns of enrichments in the N3 (78%) and N1 (53%) contained in the liver. A comparison of this data with extracts of the tumor uracil nucleotide pool demonstrated inter-organ differences i.e. N3 (48%) and N1 (7%). Secondly, in a similar study enrichments in the N1 and N3 atoms contained in the fraction of the newly formed uracil nucleotide pool of liver extracts were compared to the ^{15}N enrichment in aspartate contained in the same liver extracts. The results of this study are shown in the table:

TIME OF INFUSION (min)	%F (1)	% $^{15}N1$	% $^{15}N3$	% ^{15}N-ASP
10	1.0	77.3	68.2	11.2
45	11.4	55.8	82.0	8.3
75	16.0	59.8	80.5	9.8

(1) fraction of uracil nucelotide pool formed during the experiment

These results imply intra-organ compartmentation since the isotopic enrichment found in N1 of the newly formed uracil nucleotide pool was much higher than the enrichment quantified for the ^{15}N contained in aspartate in the same cell. The aim of this research is to verify the predictive capability of this technique by isolating an individual compartment e.g. mitochondria and to show the actual compartmentation of reaction pathway intermediates, and then to exploit the predictive nature of this technique to study the pharmacologic effects of compartmentation as it relates to antitumor activity of agents which act to inhibit pyrimidine and purine biosynthesis.

Publications:

1. Strong, J.M., Upton, D.K., Anderson, L.W., Monks, A., Chisena, C.A., and Cysyk, R.L.: A novel approach to the analysis of mass spectrally assayed stable isotope-labeling experiments. J. Biol. Chem. 260: 4276-4281, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06166-01 LBC

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Macromolecular Interactions of Vinca Alkaloids

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Ronald L. Felsted	Research Chemist	LBC, NCI
Others:	Ahmad R. Safa	Visiting Fellow	LBC, NCI
	Constance Glover	Microbiologist	LBC, NCI

COOPERATING UNITS *(if any)*

Ernest Hammel	Sr. Investigator	LMCB, NCI

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.1	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.)*

This project involves the identification of Vinca alkaloid macromolecular interactions in cells and the characterization of the relationship of these associations to overall drug anti-neoplastic activity and drug-resistance mechanisms. Radioactive photoactive analogues of vinblastine have been synthesized. The pharmacological activity of one of these analogues is compared to the parent drug. Macromolecular interactions are identified by photoaffinity labeling and by SDS-polyacrylamide gel electrophoresis. The labeled polypeptides are characterized and identified by immunoprecipitation. The mechanism of analogue binding to tubulin and inhibition of microtubule polymerization is being studied. New Vinca alkaloid binding proteins have been identified and their role in these processes is being examined. The relationship of drug-binding proteins to cellular drug-sensitivity and drug-resistance is being investigated.

The Vinca alkaloids, vinblastine and vincristine, isolated from the plant Vinca rosea L. are important chemotherapeutic agents with clinical activity against a spectrum of human cancers. It is generally assumed that the mechanism for their cytotoxic, anti-mitotic and anti-neoplastic activity is related to their binding to the tubulin dimer of microtubules with the subsequent depolymerization and disruption of the cellular microtubular network including the mitotic spindle. However, evidence from several studies suggests that binding to mitotic spindle tubulin dimers cannot account for their pronounced cytotoxic effects on slowly proliferating sensitive cells and cells in interphase since in these cells cytotoxicity is evident long before mitotic arrest becomes manifested. The Vinca alkaloids have been shown to inhibit the incorporation of ^{3}H-uridine into RNA and ^{3}H-thymidine into DNA. These effects may be due to the specific inhibition of nucleic acid synthesis as well as the inhibition of nucleotide uptake into cells. Finally, our knowledge of the mechanism involved in the expression of Vinca alkaloid resistance in cancer cells is limited. While oversynthesis of particular proteins in Vinca alkaloid resistant cells has been noted, these proteins have not been identified and their possible interactions with Vinca alkaloids have not been described.

Affinity labeling of proteins with photoactive ligands is a powerful tool in probing biological targets. The photoaffinity labeling technique allows identification of specific acceptor molecules in a complex mixture of biological components. Under photolabeling conditions a reversible complex occurs between the photoactive analogue and unique acceptor sites of specific polypeptides which recognize the characteristic drug structure. Upon irradiation with UV light, the analogue is converted into a highly reactive nitrene intermediate which then covalently labels the acceptor site.

Objectives:

This project involves the identification of specific Vinca alkaloid interactions with macromolecules in cell lines and mammalian tissues. The unique biological associations are identified by in vitro and in situ covalent labeling with radioactive photoactive vinblastine (VB) analogues. Specific radiolabeled macromolecules will be identified by immunoprecipitation. The biological and pharmacological activities of the parent VB and photoactive VB analogues will be compared. The relationship of specific Vinca alkaloid binding macromolecules to VB cytotoxic, anti-mitotic and drug-resistance mechanisms will be examined.

Major Findings:

Two photoactive VB analogues have been synthesized: (1) N-(p-azidobenzoyl)-N'-β-aminoethylvindesine (NABAV) and (2) N-(p-azidosalicilyl)-N'-β-aminoethylvindesine (NASAV). The NABAV has been prepared radioactive: N-(p-azido-3,5-[^{3}H]-benzoyl)-N'-β-aminoethylvindesine (^{3}H-NABAV). The ^{3}H-NABAV has been used to probe Vinca alkaloid macromolecular interactions by photoaffinity labeling homogenates of calf brain and tumor cell lines. After photolabeling, Vinca alkaloid binding components were resolved by SDS-PAGE and identified by comparing the radioactivity in 1 mm gel slices to polypeptide MW standards. Prominently labeled components of M_r 54.3 and 21.5 Kd corresponded to unique Coomassie blue stained polypeptides, and were superimposed upon a background of nonspecific radioactivity. The M_r 54.3

polypeptide was found in both the 100,000 x g pellet and supernatant fraction whereas the M_r 21.5 polypeptide was exclusively located in the 100,000 x g pellet. Photolabeling was maximal after 10 min UV light exposure and linear to 150 µg of protein. Photolabeling of M_r 54.3 and 21.5 with increasing concentrations of ^{3}H-NABAV (0-1.32 µM) was biphasic, consistent with mixed specific and nonspecific labeling. After correcting for nonspecific labeling, M_r 54.3 and 21.5 exhibited a saturation above 0.33 µM ^{3}H-NABAV. Increasing concentrations of non-radioactive NABAV or VB added to 0.44 µM ^{3}H-NABAV resulted in a limited reversal of photolabeling of M_r 54.3 and 21.5 corresponding to 3.2 and 0.62 pmol/mg calf brain homogenate protein, respectively, confirming the specific photoaffinity labeling of these Vinca alkaloid acceptors. Ka(VB)/Ka(NABAV) for M_r 54.3 was 0.86. The M_r 54.3 component was also prominent in human KG1 myeloid, MCF-7 breast cancer cells and P388 mouse leukemia cells. The M_r 54.3 polypeptide was identified as a tubulin subunit by immunoprecipitation in calf brain and P388 supernatant fractions.

The NABAV and VB blocked tubulin polymerization in vitro at similar concentrations. The cytotoxic concentrations of NABAV and VB toward P388 cells were identical. Thin-section electron microscopy of NABAV or VB treated P388 and MCF-7 cells revealed similar multinucleated and mitotic arrested cells and induction of tubulin crystals, annulated lamellae and vaculolized rough endoplasmic reticulum.

Proposed Course:

The above data confirm the similarities of NABAV to its parent VB and indicate its usefulness for identifying Vinca alkaloid binding proteins. These proteins may play significant roles in the mechanism of drug action. The NABAV will be used to probe for specific VB binding sites in pleiotropic drug resistant cell lines. Initially, wild-type and drug-resistant cells will be photoaffinity labeled and the radioactive labeled polypeptides will be compared in order to identify differences which might represent cellular components which play direct roles in drug resistance mechanisms. Specifically, we will examine cell lines previously shown to exhibit unique polypeptide changes proportional to the level of acquired drug resistance (i.e. CEM/VLB/100 human lymphoid cells obtained from W. Beck and the colchicine resistant Chinese hampster cells obtained from J. Biedler). We will also compare the following apparent pleiotropic cell lines: HL-60 (N. Ahmad), KB (I. Pastan) and MCF-7 (K. Cowen). Unique differences will be examined with ^{3}H-NABAV. The ^{125}I-NASAV analogue also will be used to increase the detection sensitivity.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06167-01 LBC

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Inhibition of Myristoylation-Dependent Oncogene Mediated Cellular Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.L. Felsted	Research Chemist	LBC, NCI
Other:	C. Glover	Microbiologist	LBC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Medicinal Chemistry and Pharmacology, DTP, DCT, NCI (V. Marquez); Frederick Cancer Research Facility (A. Shultz, S. Oroszlan).

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.9	0.9	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 modification of onc-proteins with the fatty acid myristate is an early step associated with the transformation of normal to neoplastic cells. The exact significance of myristoylation in transformation has not been established. However, it is thought to be part of the mechanism by which cytoplasmic oncogene kinases are localized to the inner plasma membrane surface. Since the transforming activity of onc-kinases is dependent upon this membrane binding, this project will investigate the role of myristoylation as it relates to the mechanism of this subcellular localization. A specific myristoylation assay is being developed using synthetic NH_2-terminal polypeptide homologues of cellular and viral myristoylated proteins. The myristyl transferase(s) substrate specificity for polypeptide and fatty acid will be examined in order to define the myristoylation mechanism. The role of covalently bound myristic acid in translocation of myristoylated proteins from their site of cytoplasmic synthesis to the plasma membrane will be examined using mutants of myristoyl-proteins which lack the fatty acid and with myristoyl transferase inhibitors. The involvement of myristic acid in membrane binding will be examined by looking for specific membrane receptors of the myristoyl-proteins. Information on the enzymology of myristoylation and the role of myristic acid in translocation and membrane binding will be used to design and synthesize specific inhibitors of myristoylation in these related phenomena with the goal of developing chemotherapeutic agents specific for a critical early step of malignant transformation.

Tyrosine-specific protein kinase activity is associated with several known oncogenes and is an appealing target for the chemical manipulation of kinase associated cellular transformation. Selective inhibition of onc-kinases appears to be a formidable task, but may become possible when the cellular substrate(s) for the onc-kinases are identified. Recently it has been observed that direct inhibition of the onc-kinases may not be necessary in order to block (or reverse) transformation. The transforming activity of onc-kinases depends upon their association with the inner plasma membrane surface. It has been proposed that the mechanism by which viral encoded onc-kinases such as p60src become membrane bound is through a post- or co-translational addition of myristic acid to their NH_2-terminal glycine via an amide linkage. In recently published experiments, Cross et al. constructed p60src NH_2-terminal deletion mutants that failed to incorporate myristic acid. These mutant proteins were still functional kinases in vitro and in vivo, but instead of being localized to the membrane they were found in the cytoplasm. Most pertinent to this proposal, these mutant proteins no longer transformed cells. Presumably, myristoylation is critical to p60src subcellular localization and cellular transforming activity.

Myristoylation is also necessary for the membrane localization of the cellular p60src tyrosine kinase encoded by the src proto-oncogene. In addition, several normal and neoplastic mammalian cells have elevated levels of a similar size tyrosine kinase, p58. However, this p58 kinase is distinct from the cellular and viral p60src as well as from five other proto-oncogene cellular tyrosine kinases and it does not contain gag linked viral proteins. Most interestingly, one of these p58 kinases contains myristic acid. These results indicate that myristoylation may be an important regulator of the subcellular distribution and function of normal cellular tyrosine kinases. Thus, transformation resulting from the expression of structurally modified tyrosine kinases or elevated amounts of cellular kinases also may depend on myristoylation-directed subcellular distribution.

Objectives:

In this project we will examine the role of myristoylation of onc-tyrosine kinases as targets for the chemotherapeutic inhibition of cellular transformation. Specifically, we will study three mechanisms for blocking myristoylation dependent transformation by p60src: these include, (i) inhibition of the myristoyl transferase(s), (ii) the blocking of translocation of soluble myristoyl-proteins to their membrane binding sites, and (iii) inhibition of binding of myristoyl-proteins to potential membrane receptors. We will examine, also, the relationship of myristoylation to normal tyrosine kinases and the role of myristoylated-tyrosine kinases in normal cellular growth and differentiation.

Proposed Course:

i. Myristoyl Transferase(s). Fatty acid amides are well known in nature and their syntheses by eucaryotic cell free systems have been described. Myristoyl amidation takes place during or immediately after protein synthesis so fatty acid addition may occur by a co-translational process as in the case for NH_2-terminal acetylation. The myristoyl transferase(s) also are characterized by an absolute specificity for myristic acid. The question of substrate specifi-

city will be examined in order to develop a specific enzyme assay for myristoylation. It is likely that nascent polypeptide chains are the actual substrates of the myristoyl transferase(s). Studies with p29gag-ras and deletion construct mutants of p60src indicate that the first 13-15 terminal amino acids may present a conformation-dependent myristoylation acceptor site as the substrate for myristoyl transferase(s). In collaboration with Drs. Alan Shultz and Stephen Oroszlan of the Frederick Cancer Research Facility, we will synthesize a 14-15 amino acid polypeptide corresponding to the NH_2-terminal sequence of the p60src protein. In addition, a tyrosine residue will be added to the COOH-terminal end of the synthetic polypeptide to allow for radiolabeling with ^{125}I. This NH_2-terminal glycine peptide will be tested as a substrate in a specific myristoyl transferase assay and used as a probe for myristoyl-protein acceptors (see ii and iii, below).

Synthetic polypeptides corresponding to several known myristoyl acceptor amino acid sequences will be tested to assess relative myristoyl transferase substrate specificity. Polypeptide analogues of varying lengths and with selected amino acid substitutions will be tested as competitive inhibitors. In collaboration with Dr. Victor Marquez of the Laboratory of Medicinal Chemistry and Pharmacology, DTP, DCT, NCI, we will design and synthesize polypeptide and/or fatty acid derivitives that will serve as specific irreversible (suicide) inhibitors of the myristoyl transferases(s). Also, once the fatty acid myristoyl donor has been identified, it might be possible to block myristoylation by inhibiting the de novo synthesis of the fatty acid donor.

ii. Myristoyl-protein translocation. Shortly after synthesis on free ribosomes, the myristoyl-p60src kinase forms a soluble complex with two cellular proteins of 50 and 90 kilodaltons. It has been suggested that this complex may act as a device for transporting newly synthesized p60src kinase to its site of transforming action at the plasma membrane. Similar complexes have been reported for onc-kinases encoded by other retroviruses so "complex mediated" translocation might be another important step in onc-kinase cellular transformation. Although the p50 and p90 proteins are not thought to bind to the NH_2-terminal region of p60src, the role of myristoylation in this complex is not understood. It may be possible to establish the function of myristic acid in the carrier mediated translocation by testing to see if mutants of p60src kinases which lack myristic acid are still able to form comparable complexes with p50 and p90. Ultimately, it may be necessary to use specific inhibitors of myristoyl transferases(s) (see i, above) or myristoyl-protein binding (see iii, below) in order to clarify the role of myristic acid in translocation.

iii. Myristoyl-protein membrane binding. Myristic acid may play a direct role in the binding of onc-proteins to the plasma membrane. This could occur by one of two basic mechanisms. In one mechanism, the hydrophobic fatty acid could serve as an anchor for the protein by embedding into the membrane lipid bilayer. In fact, this has been shown for some integral membrane proteins where the palmitic acid esterification of membrane bound nascent polypeptides is part of a maturing process which results in these proteins being more firmly anchored in the membrane. This anchor mechanism also is compatible with the carrier mediated translocation process described above (see ii.). Since myristoylation occurs in the cytoplasm, premature anchoring to the nearest intracellar membrane bilayer could be prevented by complexing the newly modified

onc-protein to specific carrier proteins. In turn the carrier proteins could provide the recognition signal that determines the ultimate membrane destination. However, once the complex arrives at the membrane and the carrier proteins release the transported onc-protein, membrane binding must be determined by intrinsic structural features of the myristoyl-onc-protein; in this case, myristate insertion into the membrane bilayer. The problem with this hypothesis is that in contrast to the irreversible binding of palmitoyl ester modified integral membrane proteins, myristoyl-proteins are reversibly membrane bound.

A second mechanism which could provide for reversibility would be the recognition of and binding to myristate by specific membrane receptors. If, in addition, the receptor recognizes the adjoining NH_2-terminal polypeptide sequence, then an added degree of specificity would result (i.e. the binding of p60src specifically to membrane adhesion plaques). We propose to examine the role of myristoyl-protein binding by classical receptor techniques. Using the radiolabeled myristoylated synthetic polypeptides described above, we will look for saturable binding to membrane vesicles as well as to soluble binding proteins such as the p50 and p90 (see ii, above). In addition, specificity will be confirmed by competitive displacement of membrane bound radioactive myristoyl-onc-proteins with nonradioactive synthetic peptides. This displacement assay also will be used to survey for potential inhibitors of myristoyl-protein receptor binding and/or myristoyl-protein carrier complex binding. Simple amide combinations of fatty acid and synthetic polypeptides will be examined as potential inhibitors of the translocation and membrane binding of myristoylated onc-proteins.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06168-01 LBC

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Cellular Pharmacology of Interferon and Double-stranded RNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Mrunal Chapekar	Visiting Associate	LBC, NCI
	Victor E. Marquez	Visiting Scientist	LPET, NCI

COOPERATING UNITS *(if any)*

Medicinal Chemistry Section, LPET, DTP, DCT, NCI (V. Marquez)

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

This study will entail: 1) investigations of the cyclopentenyl and 5'-phosphonate analogs of the interferon-induced 2',5'-oligoadenylate as specific inhibitors of 2',5'-oligo(A) phosphodiesterase, and 2) determination of the mechanism of the potentiation produced by poly(I)·poly(C) and its derivatives on the antitumor activity of immune interferon (IFN-γ). This will include investigating the role of transcription and cellular oncogene expression by the synergistic combination of poly(I)·poly(C) and IFN-γ in human colon carcinoma cell lines HT-29 and BE.

GPO 914-918

This area of study involves two projects. The first will examine the activity of carbocyclic and phosphonate analogs of 2',5'oligo(A), the interferon-induced oligonucleotide believed to mediate the antiviral or possibly the antiproliferative activity of interferons (Fig. 1). Dr. Chapekar's previous studies of 2',5'-oligo(A) metabolism in response to interferon and/or double-stranded RNA (poly(I)·poly(C)), showed that 2',5'-oligo(A) phosphodiesterase (PDE) is rate-limiting in degrading 2',5'oligo(A) in the cell, and in fact, none could be detected by a sensitive radioligand binding assay. Thus, in collaboration with Dr. Victor E. Marquez, Medicinal Chemistry Section, LPET, we plan to investigate whether 5'-phosphonate (1) or neplanocin A (2) analogs of 2',5'-oligo(A) are specific, and possibly irreversible inhibitors of 2',5'-oligo(A) phosphodiesterase (Fig. 1), and thus lead to potentiation of the activity of interferon.

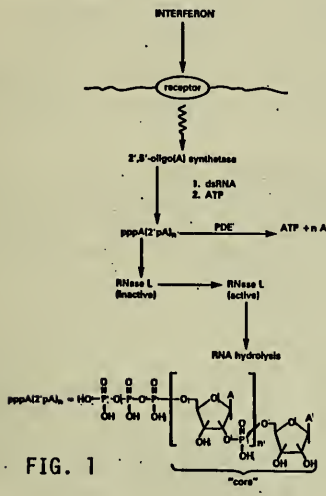
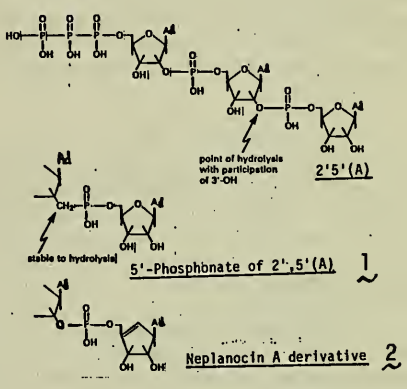


FIG. 1



Studies over the past three years in this Section have involved an examination of the mechanism of action of human leukocyte, fibroblast and immune interferons-α, β and γ (IFN-α, β and γ). These investigations showed that IFN-α and -β interferons were not highly cytocidal to HT-29 cells in vitro, and were in fact, inactive against several human leukemias. Coadministration of poly(I)·poly(C) moderately increased cytotoxicity but it was only an additive effect. Further studies with natural and recombinant IFN-γ have established that this lymphokine is a potent inhibitor of cell proliferation as well as being highly cytocidal. Three day treatment of HT-29 cells with 200 units/ml of IFN-γ resulted in 90% growth inhibition, and 99% reduction in colony formation.

The antitumor effects of IFN-γ were further explored in combination with poly(I)·poly(C). Unlike combinations of IFN-α and IFN-β with this double-stranded RNA, IFN-γ and poly(I)·poly(C) showed a syntergistic effect on cell viability in colon carcinoma cell lines HT-29 and BE. Three day treatment with 10-25 units/ml of IFN-γ resulted in 30-40% reduction in cell viability in HT-29 cells and a 10% reduction in BE cells. Coaddition of poly(I)·poly(C) with IFN-γ resulted in a 70-90% reduction in colony formation in HT-29 cells and a 50-60% reduction in BE cells. Most notable was the appearance of this effect in cell line BE which is resistant to either drug alone. The appearance of the synergistic effect on cell viability was accompanied by a corresponding reduction in transcription, but not by rRNA degradation, changes in 2', 5'-oligo(A) levels or alterations in protein phosphorylation in vitro. These results establish a clear association between RNA transcription and cell lethality. Thus, the second area of study related to the scheme in Fig. 1 is the mechanism of the potentiation of interferon activity by double-stranded RNA (dsRNA). The dsRNAs being evaluated are poly(I)·poly(C), poly(I)·poly(C)·polylysine·-carboxymethylcellulose (ICLC) and mismatched poly(I)·poly(C) where there is a 13% substitution of uridylic acid in the poly(C) (also called "Ampligen", Hem Research Inc.). Since IFN-γ, ICLC and "Ampligen" are undergoing phase I clinical trials, it will be important to assess the efficacy of these dsRNAs for potentiating the cytocidal effect of IFN-γ.

Our data imply that there is a very sensitive dsRNA-regulated component to transcription and we plan to investigate this phenomenon by measuring the transcription of cellular oncogenes believed to be involved in cell cycle transit. Studies of this nature in human colon carcinoma cell lines HT-29 (IFN-γ sensitive) and BE (IFN-γ refractory) are currently in progress.

Major Findings:

1. The effect of polyinosinic·polycytidylic acid (poly(I)·poly(C)) on the cell lethality produced by 5-fluorouracil (FUra) and 5-fluorouridine (FUrd) was examined in human colon carcinoma cell line HT-29. Pretreatment of cells with poly(I)·poly(C), as well as during exposure to FUra or FUrd resulted in antagonism of the cell lethality generated by the fluoropyrimidines. Antagonism of FUra cytotoxicty was also produced by the 2'-O-methylated analog, poly(I)·poly(C_m), but not by the individual single-stranded polynucleotides or by the component mononucleotides, CMP and IMP. In contrast, CMP blocked the toxicity of FUrd. The antagonism by poly(I)·poly(C) of FUra and FUrd cytotoxicity was related to the inhibition of their metabolism to FUTP and their incorporation into RNA and not to inhibition of the synthesis of RNA. Antibodies to leukocyte and fibroblast interferons did not reverse the antagonistic activity of poly(I)·poly(C). These results indicate that poly(I)·poly(C) may be interfering with the transport and/or initial metabolism of FUra and FUrd to FUMP which is independent of the ability of the double-stranded RNA to induce interferon. (Iigo and Glazer, Cancer Res. 45: in press).

2. The effect of polyriboinosinic · polyribocytidylic acid (poly(I)·poly(C)) on the antitumor activity of 5-fluorouracil (FUra) and 5-fluorouridine (FUrd) was evaluated in mice bearing L1210 leukemia. Coadministration intravenously of poly(I)·poly(C) and either FUra or FUrd on days 1, 5 and 9 to mice-bearing L1210 leukemia implanted subcutaneously resulted in a 40% greater increase

in lifespan at the optimal antitumor dose vs. FUra or FUrd alone. This effect appeared to result from greater host tolerance of a dose of FUra or FUrd which would otherwise be cytotoxic. The protective effect of poly(I)·poly(C) was also evident in non-tumor-bearing mice as well as following administration of drug intraperitoneally to mice bearing the tumor implanted intraperitoneally. FUrd incorporation into RNA in the spleen, bone marrow and small intestine revealed little or no changes after coadministration of poly(I)·poly(C). (2',5')Oligo(A) synthetase activity, an indication of interferon activity, was markedly depressed in the spleen and bone marrow following treatment with FUrd; however, poly(I)·poly(C) administered together with FUrd returned (2',5')oligo(A) synthetase activity to normal levels. These data indicate that poly(I)·poly(C) ameliorates the host toxicity of fluoropyrimidines, possibly via an interferon-mediated effect, and thereby results in enhanced therapeutic efficacy of the antimetabolite as an antitumor agent. (Iigo and Glazer, Cancer Res. 45: in press, 1985).

3. The cytocidal activity of human immune interferon (IFN-γ) in combination with the synthetic double-stranded RNA, poly(I)·poly(C), was investigated in human colon carcinoma cell line HT-29. Three day treatment with 10 to 25 units/ml of IFN-γ resulted in 30 to 40 % reduction in colony formation, whereas 25 to 100 μg/ml of poly(I)·poly(C) reduced cell viability by 10 to 20% of control. The lethal effect of the combination of IFN-γ and poly(I·-poly(C) was synergistic wherein 70 to 90% reduction in colony formation was observed. Measurements of DNA, RNA and protein synthesis after IFN-γ and poly(I)·poly(C) treatment showed a dose-dependent reduction in all three parameters. Recombinant IFN-γ in combination with poly(I)·poly(C) exhibited a similar effect. Studies evaluating the molecular mechanism of IFN-γ and poly(I)·poly(C) toxicity indicate a lack of involvement of the double-stranded RNA-dependent (2',5')oligo(A)-RNAse L and protein kinase pathways; however, the effect appears to be related to the inhibition of ribosomal RNA transcription in this cell line. (Chapekar and Glazer, Cancer Res. 45: in press).

Publications:

1. Iigo, M. and Glazer, R.I.: Antagonistic effect of polyinosinic-polycytidylic acid on cell lethality produced by 5-fluorouracil in human colon carcinoma cells in vitro. Cancer Res. 45: 1953-1957, 1985.

2. Iigo, M., Chapekar, M.S. and Glazer, R.I.: Synergistic antitumor effect of fluoropyrimidines and polyinosinic-polycytidylic acid against L1210 leukemia. Cancer Res., in press.

3. Chapekar, M.S. and Glazer, R.I.: Synergistic effect of human immune interferon and double-stranded RNA against human colon carcinoma cells. Cancer Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06169-01 LBC

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular and Molecular Pharmacology of Phospholipid and Diacylglycerol Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Kathleen D. Hartman	Chemist	LBC, NCI
	Marion C. Knode	Biologist	LBC, NCI
	Ester Zylber-Katz	Visiting Scientist (DTP Exchange Program)	LBC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
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SECTION

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.75	0.75	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.)*

Dialkylphospholipid and dialkylglycerol analogs will be investigated in human promyelocytic leukemia cell line HL-60. These studies will include examining the role of phosphatidylinositol metabolism and the cellular oncogenes c-myc, c-H-ras and c-fos in the differentiative and antiproliferative effects of these analogs. The compounds 1-O-hexadecyl-2-O-methyl-3-phosphocholine and 1-O-hexadecyl-2-O-methyl-3-phosphate are currently being evaluated.

GPO 914-918

One of the most intensively investigated areas of cell biology in the past few years is the process of calcium- and phospholipid-dependent protein phosphorylation (Fig. 1). Inducers of the protein kinase activity (protein kinase C) believed to be responsible for this process, such as diacylglycerols(1) and phorbol esters(2), have shown the importance of this enzyme in the regulation of growth factor activity, lymphoblastogenesis, normal cell proliferation, cellular differentiation and oncogene expression. Studies in this laboratory of protein kinase C activity during differentiation of human promyelocytic leukemia cell line HL-60 have indicated that not only is this enzyme activity increased in cells induced to differentiate along the myeloid or monocytic pathways, but that there is the rapid appearance of differentiation specific peptides which are phosphorylated in a calcium- and phospholipid-dependent manner.

In association with the activation of protein kinase C activity is the turnover of phospholipids such as phosphatidylinositol-4,5-bisphosphate(3) to yield diacylglycerol and inositol phosphates, which in turn activate phosphorylation (Fig. 1). In addition, inositol phosphates are involved in the mobilization of calcium within the cell and thus set in motion calcium-dependent protein phosphorylation as well. One series of natural metabolites known to

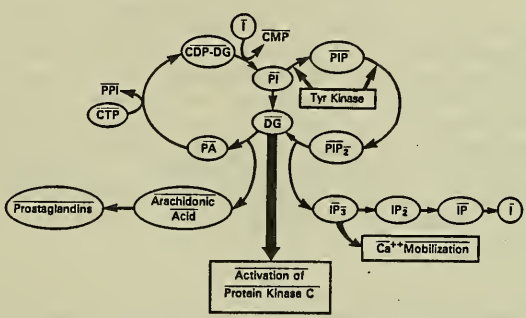
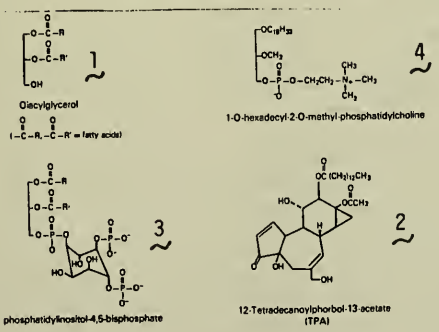


FIG. 1



produce differentiating activity and activation of protein kinase C and phosphatidylinositol turnover are the diacylglycerols. Related dialkylglycerols are more metabolically stable and more effective inducers of differentiation in HL-60 cells. Moreover, phospholipid analogs such as dialkylphosphatidylcholine(~) are also selective cytotoxic drugs against HL-60 cells vs. normal neutrophils and in some instances possess differentiating activity as well.

Thus, it is proposed that dialkylglycerol and dialkylphospholipid analogs be investigated for their potential as selective anticancer drugs via their capacity to mobilize calcium- and phospholipid-dependent processes related to cellular differentiation. We are evaluating 1-O-hexedecyl-2-O-methyl phosphatidylcholine(~) and its corresponding phosphatidic acid derivative (C-16-OCH_3PA). The dialkyl PC analog was originally synthesized as a derivative of platelet activating factor but was inactive as such due to the C-2 ether linkage. However, the drug was found to have preferential toxicity against HL-60 leukemia cells vs. normal neutrophils where the IC_{50} for HL-60 cells was about 50-fold less than for neutrophils. The other analog, C-16-OCH_3PA, is a soluble form of dialkylglycerol and is less cytotoxic to HL-60 cells. However, it induces monocytic differentiation in this cell line to a greater extent than C-16-OCH_3PC and may resemble more closely the action of diacylglycerol and phorbol esters in inducing differentiation of HL-60 cells along the monocyte-macrophage pathway.

Thus, we plan to examine perturbation of phosphatidylinositol metabolism and cellular oncogene expression in HL-60 cells in response to C-16-OCH_3PC and C-16-OCH_3PA. Other analogs will be synthesized in collaboration with Dr. Joseph Hajdu, California State University, and include 1-O-hexadecyl-2-acetamide-phosphatidylcholine and 1-O-hexadecyl-2-acetamido glycerol. We also propose to have synthesized dialkyl analogs of phosphatidylinositol, and possibly analogs modified in the inositol moiety. The latter compounds in particular, may serve as novel compounds for the delivery of inositol analogs into the cell since inositol itself is poorly transported.

Major Findings:

The effects of differentiating agents on the activity and phosphorylation pattern produced by phospholipid- and Ca^{++}-dependent protein kinase (PL-Ca-PK) was examined in human promyelocytic leukemia cell line HL-60. Dimethylsulfoxide (DMSO), retinoic acid (RA) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) increased the appearance of mature myelocytic (DMSO and RA) or monocytic ($1,25(OH)_2D_3$) cells. The tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) increased the appearance of adherent macrophage-like cells. Coincident with the appearance of differentiated cells induced by DMSO, RA and $1,25(OH)_2D_3$ was an increase in PL-Ca-PK activity. In contrast, TPA treatment resulted in the rapid disappearance of PL-Ca-PK and the induction of PL-Ca-independent protein kinase activity. The phosphorylation pattern resulting from endogenous PL-Ca-PK in extracts from cells treated with DMSO, RA or $1,25(OH)_2D_3$ showed a prominent phosphorylated protein of 37 kilodaltons (pp37) and 38 kilodaltons (pp38) which was related to the appearance of the myelocyte/monocyte phenotype. pp37 and pp38 were also present in TPA-treated cells but their phosphorylation was no longer dependent on the presence of PL and Ca. Cells treated with DMSO and RA also exhibited a PL-Ca-dependent pp21 which was barely evident in $1,25(OH)D_3$-treated cells, and thus, represented a myeloid cell marker. Also

present was a prominent PL-Ca-dependent pp19 which remained unchanged following treatment with DMSO, RA and $1,25(OH)_2D_3$, but markedly diminished in TPA-treated cells. On the other hand, TPA-treated cells exhibited a characteristic pp130 which was antigenically related to the actin binding protein, vinculin. These results indicate that there are characteristic PL-Ca-dependent phosphorylated proteins indicative of mature myelocytic and monocytic cells, as well as PL-Ca-independent phosphorylated proteins characteristic of the macrophage-like phenotype. (Zylber-Katz & Glazer, Cancer Res. in press.)

Publications:

1. Zylber-Katz, E. and Glazer, R.I.: Phospholipid- and calcium-dependent protein kinase activity and protein phosphorylation in human promyelocytic leukemia cell line HL-60. Cancer Res., in press.

2. Zylber-Katz, E. and Glazer, R.I.: Cooperative effects of retinoic acid and dimethyl sulfoxide on phorbol ester-mediated differentiation and phospholipid- and calcium-dependent protein phosphorylation in human promyelocytic leukemia cell line HL-60. F.E.B.S. Letters, in press.

3. Glazer, R.I.: Differentiation of malignant cells as a new mode of chemotherapy. In: Elsebai I. (Ed.): Current Treatment of Cancer. Heidelberg, VICC-Springer-Verlag, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07109-09 LBC

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Molecular and Cellular Pharmacology of Nucleoside Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Marvin B. Cohen	Staff Fellow	LBC, NCI
	Kathleen D. Hartman	Chemist	LBC, NCI
	Marion C. Knode	Biologist	LBC, NCI
	Kasturi Sriram	Visiting Fellow	LBC, NCI
	Victor E. Marquez	Visiting Scientist	LPET, NCI

COOPERATING UNITS *(if any)*

Medicinal Chemistry Section, LPET, DTP, DCT, NCI (V. Marquez)

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

The mechanism of action of nucleoside antimetabolites will be assessed in human tumor cells in tissue culture, and will include studies of ribosomal RNA processing, transcription, methylation of RNA and DNA and cellular oncogene expression. These studies will deal with the cyclopentenyl analogs of adenosine (neplanocin-A), cytidine, uridine, 3-deazaadenosine 5-fluorouridine and 5-azacytidine. The activity of these drugs will be assessed by the clonogenic survival of human colon carcinoma cell line HT-29 and the differentiation response of human promyelocytic leukemia cell line HL-60.

PHS 6040 (Rev. 1/84) GPO 914-918

Molecular and Cellular Pharmacology of Anticancer Drugs. The area of research most intensively pursued over the past seven years by this Section has been the mechanism of action of adenosine analogs and pyrimidine antimetabolites. The newest compounds related to these anticancer drugs are the cyclopentenyl series typified by the adenosine analog, neplanocin A (1). This drug is unique that it is the only nucleoside analog which appears to act as a specific inhibitor of RNA methylation via its anabolism to a metabolite of S-adenosyl-L-methonine (Fig. 1) without itself being significantly incorporated into RNA. It is proposed that other purine analogs in this series will yield equally interesting biological activities. For example, it is expected that the cyclopentenyl analog of 3-deazaristeromycin (3-deazacarbocyclic adenosine) will act as an irreversible inhibitor of S-adenosylhomocysteine hydrolase (Fig. 1). Moreover, cyclopentenyl analogs of pyrimidine nucleosides have already yielded a nontoxic inhibitor of cytidine-uridine kinase (cyclopentenyluridine) and a highly potent and irreversible inhibitor of cytidine triphosphate synthetase (cyclopentenylcytidine) (2).

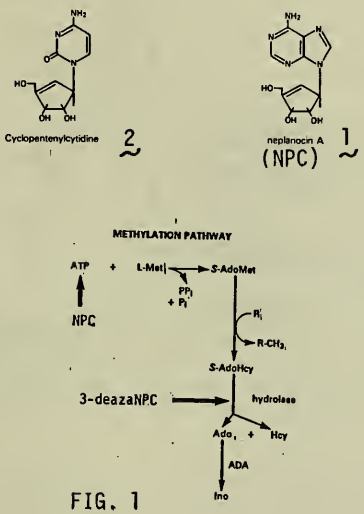


FIG. 1

It is expected that the corresponding analogs of 5-fluorouridine and 5-azacytidine will also yield unique and perhaps more selective cytotoxic and pharmacological activities. Therefore, this laboratory in collaboration with the Medicinal Chemistry Section, LPET, will continue to investigate the mechanism of action as well as the cytocidal activity of these drugs in human tumor cell lines such as colon adenocarcinoma HT-29, the tissue culture counterpart of the colon tumor xenograft used for drug screening. In addition to this tissue culture line, we will also assess the effect of these agents on the human promyelocytic leukemia cell line HL-60. This cell line has the ability to

differentiate along the myelo- or monocytic pathways in response to a diverse group of compounds. Thus far, our studies with the cyclopentenyl analogs have demonstrated that cyclopentenyl cytidine is a potent and rapid inducer of myeloid differentiation in HL-60 cells with activity superior to that of retinoic acid, and that neplanocin A produces a partial myelocytic response. Having drugs with precise mechanisms of action may help to elucidate the key cycle phase specific events required to induce differentiation. This approach is being utilized by measuring the expression of various cellular (proto) oncogenes believed to be involved in cellular proliferation and differentiation (Fig. 2).

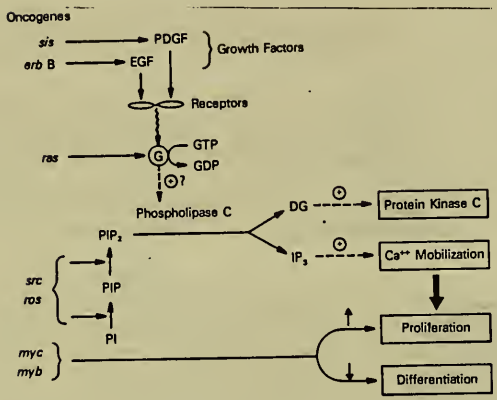


FIG. 2

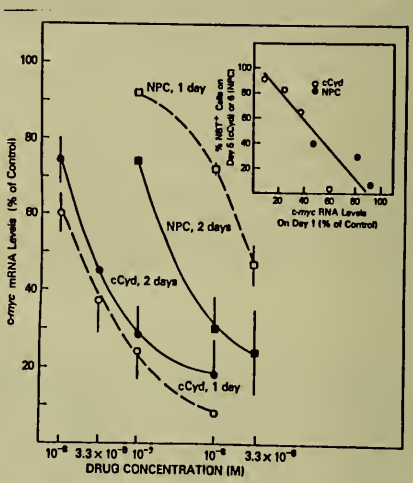


FIG. 3

It appears that for c-myc mRNA, there is a striking correlation between the degree of differentiation on day 5 or 6 and the reduction in c-myc mRNA levels 1 hour after drug treatment (Fig. 3). This suggests that suppression of transcription of c-myc mRNA is a prerequisite for differentiation to occur.

Since cyclopentenylcytidine is the most potent inhibitor of DNA synthesis and c-myc RNA synthesis, as well as the most effective and rapid inducer of

differentiation among the carbocyclic analogs thus far, these data suggest that c-myc RNA synthesis occurs in S phase and that accumulation of cells in the G1 phase is a prerequisite for differentiation to proceed. Other cellular oncogenes such as c-fos and c-H-ras are also believed to be involved in cell cycle progression. The H-ras oncogene codes for a GTP-binding protein believed to be involved in the regulation of phosphatidyl inositol metabolism, whereas the c-myc and c-fos genes code for nuclear DNA-binding proteins. Thus, we plan to examine the role of the mRNAs of these cellular oncogenes in differentiation in response to not only nucleoside analogs but other agents thought to perturb phosphatidylinositol metabolism such as analogs of phospholipids and diacylglycerol (see project "Cellular and Molecular Pharmacology of Phospholipid and Diacylglycerol Analogs").

Major Findings:

I. Nucleoside Analogs and Cellular Oncogene Expression:

1. The mechanism of action of the adenosine analog, neplanocin A (NPC) was investigated in human colon carcinoma cell line HT-29. Cell viability was reduced to 38 and 17% of control by 24 hr exposure to 10^{-5} and 10^{-4} M NPC, respectively. Cytocidal activity was not affected by inhibition of adenosine deaminase with 2'-deoxycoformycin. Concomitant with decreased cell viability was the reduced incorporation of [^{14}C]dThd and [^{3}H]Leu, and to a lesser extent [^{3}H]Urd, into acid-precipitable material. Labeling of rRNA and tRNA during drug treatment for 24 hr with [^{3}H-methyl]Met and [^{14}C]Urd revealed that NPC primarily inhibited RNA methylation, and to a lesser extent, RNA synthesis. RNase T2 digests of total RNA indicated that base and 2'-O-methylation were inhibited to approximately the same degree. Metabolites of NPC were measured by reverse-phase HPLC and it was found that the major drug metabolite was the drug analog of S-AdoMet with little formation of the respective, S-AdoHcy metabolite. NPC was utilized to a very small degree for RNA synthesis where only 2 and 30 pmoles of NPC per A_{260} were incorporated into rRNA and tRNA after 24 hr exposure to 10^{-5} and 10^{-4} M NPC, respectively. These results indicate that NPC is metabolized to a metabolite of S-AdoMet which is a poor methyl donor for RNA methyltransferases, and that the accompanying decrease in RNA methylation and protein synthesis appears to be related to its cytocidal activity. (Glazer & Knode, J. Biol. Chem. 259: 12964, 1985).

2. The mechanism of action of the cyclopentenyl analogue of cytidine, cCyd, was investigated in human colon carcinoma cell line HT-29. Upon exposure of cells to 10^{-6} M cCyd, cell viability was reduced to 20% of control, whereas cytocidal activity was not present after 2 hr of drug exposure. Cell lethality was partially reversible by Urd, Cyd or dCyd at 10^{-6} M cCyd, and fully reversible by these nucleosides at 2.5×10^{-7} M cCyd. The incorporation of [^{14}C]dThd and [^{3}H]Urd into DNA and RNA was inhibited by 50% by exposure for 2 hr to 2.5×10^{-7} and 1.5×10^{-6} M cCyd, respectively. Upon 24 hr of drug exposure, the IC_{50} for RNA synthesis was reduced 2.5-fold whereas DNA synthesis was almost totally inhibited. cCyd produced a rapid and preferential reduction of CTP synthesis with a half life of 1 hr at 10^{-6} M drug. The IC_{50} of cCyd for reducing CTP concentrations after 2 hr of drug exposure was 4×10^{-7}M. Concomitant with the reduction of CTP levels was the inhibition of transcription of rRNA, and to a lesser extent, tRNA, without changes in

the processing of nucleolar RNA. No changes in the size of DNA were produced following treatment with cCyd. These results indicate that cCyd is a potent and rapid inhibitor of CTP synthesis and that this effect correlates with its cytocidal activity. (Glazer, Cohen, Hartman, Knode, Lim and Marquez, Biochem. Pharmacol., in press).

3. The effect of the cyclopentenyl adenosine analogue, neplanocin A (NPC), on cell growth and differentiation was examined in the human promyelocytic leukemia cell line HL-60. Continuous exposure of HL-60 cells to 0.1 - 3.3 μM NPC resulted in a progressive reduction in cell growth which was accompanied by an increase in differentiation to cells with a myelocyte and neutrophil morphology. The latter effect was expressed as an increase in the capacity of cells to reduce nitroblue tetrazolium and reached a level of 40% of the total cell population. Preceding the phenotypic changes was the inhibition of RNA and DNA methylation in preference to inhibition of their synthesis which coincided with the formation of a metabolite of NPC with the chromatographic characteristics of S-adenosyl-L-methionine (AdoMet). In addition, c-myc mRNA expression, which is amplified in HL-60 cells, was markedly reduced following NPC treatment. These results indicate that NPC is an effective inhibitor of RNA and DNA methylation resulting from its conversion to an analogue of AdoMet, and that these effects appear to be responsible for reduced c-myc RNA expression and the induction of myeloid differentiation in this cell line. (Linevsky, Cohen, Hartman, Knode and Glazer, Mol. Pharmacol., in press).

4. The effects of the cyclopentenyl (cCyd) and cyclopentyl (carbodine) analogues of cytidine on differentiation, and nucleic acid and nucleotide biosynthesis was examined in human promyelocytic leukemia cell line HL-60. Continuous exposure for 5 days to 10^{-8} to 10^{-6} M cCyd or 10^{-6} to 10^{-5} M carbodine produced progressive inhibition of cell growth. During this exposure interval, pronounced differentiation to mature myeloid cells occurred wherein 95% of the cell population reduced nitroblue tetrazolium four days after exposure to 10^{-7} M cCyd or 10^{-5} carbodine. Preceding differentiation was the inhibition of DNA synthesis which reached 10% of control levels 24 hr after exposure to 10^{-7} M cCyd or 10^{-5} M carbodine, while RNA synthesis was inhibited to a lesser extent. The appearance of mature myeloid cells by cCyd was preceded by the inhibition of c-myc mRNA synthesis which was more pronounced than the reduction in total cellular RNA synthesis. These biochemical effects were accompanied by a rapid and pronounced inhibition in the synthesis of CTP, but not of UTP, ATP or GTP, where the half-life for the disappearance of CTP was 1.5 to 2 hr. Following drug removal, cells treated with cCyd showed sustained inhibition of CTP synthesis, whereas cells treated with carbodine showed almost complete recovery of CTP levels within 48 hr. These results indicate that inhibition of CTP synthetase leads to rapid inhibition of DNA and c-myc mRNA synthesis which precedes the appearance of differentiated cells, and that the cyclopentenyl analog, in contrast to the cyclopentyl derivative of cytidine leads to irreversible inhibition of CTP synthesis. (Glazer, Cohen, Hartman, Knode, Lim and Marquez, Biochem. Pharmacol., in press).

5. The effects of the pyrrolopyrimidine antibiotics, sangivamycin and toyocamycin, on the synthesis of RNA and protein, ribosomal RNA processing, and cell viability were examined in colon carcinoma cell line HT-29. Exposure for 24 hr to toyocamycin caused an exponential type of cell lethality resulting in a 4 log reduction of cell viability, while sangivamycin produced a gradual and

self-limiting type of cell lethality resulting in a 1 log reduction of cell viability. Toyocamycin at a concentration of 1 μM produced total cessation of precursor rRNA processing, while 10 μM sangivamycin produced little or no effect on processing. On the contrary, sangivamycin caused a significant decrease in protein synthesis after 6 hr, while toyocamycin had less effect. The inhibition of protein synthesis by sangivamycin results from an inhibition of the formation of complexes essential to the initiation of protein synthesis. The results suggest that the mechanism of action of these closely related agents are quite distinct. The marked loss of cell viability caused by toyocamycin correlates with its effect on rRNA processing while the slow inhibition of protein synthesis appears to be secondary to the loss of ribosome synthesis. On the other hand, the lesser cytotoxicity produced by sangivamycin results from a more direct effect on protein synthesis. Importantly, cells are much less capable of resuming normal proliferative activity after 24 hr of impaired rRNA processing than after a similar interval of reduced protein synthesis. (Cohen and Glazer, Mol. Pharmacol. 27: 349, 1985).

6. The effects of six nucleoside and base analogs, 5-fluorouracil, 5-azacytidine, sangivamycin, toyocamycin, 8-azaguanine, and tubercidin, on ribosomal RNA processing and cell viability were examined in the colon carcinoma cell line HT-29. Exposure of HT-29 cells to various concentrations of each of these compounds for 24 hr produced two distinct types of results. Toyocamycin, 5-fluorouracil, and tubercidin caused an exponential type of cell lethality resulting in 3-4 log reduction of cell viability, while sangivamycin, 8-azaguanine and 5-azacytidine produced a gradual and self-limiting type of cell lethality resulting in no greater than a 1 log reduction of cell viability. Likewise, the effects of these drugs on rRNA processing resulted in their classification into two groups: toyocamycin, 5-fluorouracil, and tubercidin caused an abnormal accumulation of the 45S precursor to rRNA, while sangivamycin, 8-azaguanine, and 5-azacytidine did not cause an accumulation of 45S RNA. Sangivamycin, 8-azaguanine and 5-azacytidine all produced an inhibitory effect on protein synthesis,while tubercidin inhibited protein synthesis at a concentration similar to that which caused the accumulation of 45S RNA, and toyocamycin and 5-flurouracil had no effect on protein synthesis at concentrations at which 45S RNA accumulated. These results show that cells are much less capable of resuming normal proliferative activity after exposure to drugs which caused the accumulation of 45S rRNA precursor, in comparison to nucleoside or base analogs which act by other mechanisms. (Cohen and Glazer, Mol. Pharmacol. 27: 308, 1985).

Publications:

1. Glazer, R.I. and Knode, M.C.: Neplanocin A: a cyclopentenyl analog of adenosine with specificity for inhibiting RNA methylation. J. Biol. Chem. 259: 12964-12969, 1984.

2. Zwelling, L.A., Minford, J., Nichols, M., Glazer, R.I. and Shackney, S.: Enhancement of intercalator-induced deoxyribonucleic acid scission and cytotoxicity in murine leukemia cells treated with 5-azacytidine. Biochem. Pharmacol. 33: 3903-3906, 1984.

3. Cohen, M.B. and Glazer, R.I.: Cytotoxicity and inhibition of ribosomal RNA processing in human colon carcinoma cells. Mol. Pharmacol. 27: 308-313, 1985.

4. Chapekar, M.S., Bustin, M. and Glazer, R.I.: Evidence that high mobility group protein 17 is not phosphorylated in human colon carcinoma cells. Biochim. Biophys. Acta 838: 351-354, 1985.

5. Cohen, M.B. and Glazer, R.I.: Comparison of the cellular and RNA-dependent effects of sangivamycin and toyocamycin in human colon carcinoma cells. Mol. Pharmacol. 27: 349-355, 1985.

6. Cohen, M.B. and Glazer, R.I.: Inhibition of interleukin-2 messenger RNA in mouse lymphocytes by 2'-deoxycoformycin and adenosine metabolites. Annals N.Y. Acad. Sci., in press, 1985.

7. Glazer, R.I., Knode, M.C., Lim, M.-I. and Marquez, V.E.: Cyclopentenyl cytidine analogue: an inhibitor of cytidine triphosphate synthesis in human colon carcinoma cells. Biochem. Pharmacol., in press.

8. Linevsky, J., Cohen, M.B., Hartman, K.D., Knode, M.C. and Glazer, R.I.: Effect of neplanocin A on differentiation, nucleic acid methylation and c-myc mRNA expression in human promyelocytic leukemia cells. Mol. Pharmacol., in press.

9. Glazer, R.I., Cohen, M.B., Hartman, K.D., Knode, M.C., Lim, M.-I. and Marquez, V.E.: Induction of differentiation in human promyelocytic leukemia cell line HL-60 by the CTP synthetase inhibitor, cyclopentenyl cytidine analogue. Biochem. Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07156-02 LBC

PERIOD COVERED
October 1, 1984 to September 30, 1985

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

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore R. Breitman	Chemist	LBC, NCI
Other:	Masue Imaizumi	Visiting Fellow	LBC, NCI
	Jiro Uozumi	Visiting Fellow	LBC, NCI
	Sharon Orndorf	Microbiologist	LBC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	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 availability of tissue culture cell lines has made it possible to study the regulation of proliferation and differentiation of specific hematopoietic cell types and the effects on these cells of known or suspected mediators and modulators. It was found previously in this laboratory that retinoic acid (RA) is a potent inducer of terminal differentiation of the human promyelocytic cell line, HL-60, and the human monoblast- and monocyte-like cell lines, U-937 and THP-1. In addition RA was found to induce differentiation of fresh cells in primary culture of patients with acute promyelocytic leukemia. While retinoic acid alone is capable of inducing terminal differentiation, combinations of a physiological concentration of RA (10 nM) and either cAMP inducing agents (e.g., cAMP, prostaglandin E, or cholera toxin) or the conditioned medium from either activated T-cells or human leukemic T-cell lines synergistically induced differentiation of HL-60. An activity called "differentiation inducing activity" or DIA has been purified from these conditioned media and attempts are now being made to produce monoclonal antibodies against this protein. In addition to DIA, gamma-interferon also has differentiation inducing activity. However, polyclonal antibody against gamma-interferon does not neutralize DIA. Studies are now in progress to apply the results obtained in vitro with HL-60 to a transplantable HL-60 tumor carried in athymic nude mice. These studies should have applicability in the treatment of patients with some leukemias.

Objectives:

This project seeks an understanding of the process of terminal differentiation of myeloid cells. It is now generally agreed that some leukemias, as well as other malignancies, are diseases resulting from a block in terminal differentiation. This view suggests that viable treatment may be possible with agents that induce differentiation. To aid in this search, studies are conducted to: a) better understand the mechanism(s) of terminal differentiation; b) study the metabolism of known inducers of differentiation, e.g., RA, to aid in the development of more potent inducers; 3) screen known and newly synthesized compounds for their differentiation inducing activity; d) employ an animal model system (transplantable HL-60 carried in athymic nude mice) to determine if inducers of differentiation in vitro are active by a similar mechanism in vivo; e) establish as transplantable tumors in athymic nude mice other human myeloid leukemia cell lines to be used for testing protocols developed for treating the HL-60 transplantable tumor line.

Methods Employed:

The principal methods employed involve measurement of differentiation of human leukemia cell lines in cell culture. Most studies are conducted with the HL-60 promyelocytic cell line. Differentiation is assessed primarily by morphology and the ability of cells to reduce nitroblue tetrazolium to a formazan after incubation of HL-60 cells for 4-5 days in the presence of an inducer.

Major Findings:

1. Combinations of DIA and a physiological concentration of RA (10 nM) induce HL-60 to differentiate to cells that have monocytic markers and that can carry out several important functions of phagocytes including immunophagocytosis, development of receptors for the chemotactic peptide FMLP, and chemotaxis. In contrast, HL-60 induced with RA alone do not develop chemotactic peptide receptors and do not exhibit chemotaxis. These results suggest that therapy in vivo with RA as a sole agent may not be as effective as a combination therapy with DIA (or possibly other lymphokines such as gamma-interferon) and RA.

2. A polyclonal antibody against gamma-interferon did not neutralize the activity of a more purified DIA. This result indicates that DIA is distinct from gamma-interferon even though gamma-interferon is a DIA.

Proposed Course:

1. A large effort is underway to produce a monoclonal antibody to DIA. This antibody will be used to purify large quantities of DIA by affinity chromatography so that amino acid sequence data can be obtained. This information will allow us to construct the necessary probes for the isolation of the gene and the cloning of this potentially important product.

2. Purified DIA will be labeled with ^{125}I and studies conducted on its probable binding to specific receptors on responsive cells.

3. The transplantable HL-60 tumor line will be used to determine if substances that induce differentiation in vitro also induce differentiation in vivo. In

addition, attempts will be made to establish as transplantable tumors other leukemia cell lines such as KG-1, THP-1, and K-562.

4. Studies will be conducted on the mechanism of RA-induction of HL-60. Initially, efforts will be directed to determine if a stable covalent bond is formed between the retinoyl moiety of RA and a protein(s) in a manner similar to fatty acid-acylation of proteins.

Publications:

1. Hemmi, H. and Breitman, T.R. Induction of functional differentiation of a human monocytic leukemia cell line (THP-1) by retinoic acid and cholera toxin. JPN. J. Cancer Res. (Gann) 76: 345-351 (1985).

2. Breitman, T.R., Keene, B.R. and Hemmi, H.: Growth and differentiation of human myelomonocytic leukemia cell lines in serum free medium. In Mather, J. (Ed.): Mammalian Cell Culture. The Use of Serum-Free and Hormone Supplemented Media. New York, Plenum Press, 1984, in press.

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4. Hemmi, H. and Breitman, T.R.: Induction of differentiation of the human promyelocytic cell line HL-60 and primary cultures of human leukemia cells. A model for clinical treatment. In Glazer, R.I. (ED.) Developments in Cancer Chemotherapy. Boca Raton, FL, CRC Press, 1984, in press.

5. Imaizumi, M. and Breitman, T.R. Retinoic acid-induced differentiation of the human promyelocytic leukemia cell line, HL-60, and fresh human leukemia cells in primary culture: a model for differentiation inducing therapy of leukemia. Leukemia Reviews Int., in press.

ANNUAL REPORT OF THE LABORATORY OF EXPERIMENTAL THERAPEUTICS AND METABOLISM

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

The Laboratory of Experimental Therapeutics and Metabolism (LETM) personnel conduct experiments and generate basic information that contributes to the improvement of cancer treatment. This interdisciplinary research program in tumor biology, pathology, biochemistry, pharmacology and toxicology has overall aims that include 1) utilization of the biochemical and pathobiological characteristics of hepatic and extrahepatic tumor cell populations to define the histogenesis of specific cancers; 2) establishment of specific animal and human tumor cell lines to study new and existing anticancer drugs for their mechanism of toxicity; 3) investigation of biochemical and molecular mechanisms of drug metabolism and drug toxicity; 4) elucidation of the metabolic and physiological factors that may underlie target organ and target cell specific toxicity and prevention of toxicity by cellular defense mechanisms and 5) the continuation of the development of suitable cellular and animal models to elucidate mechanisms of toxicity of anticancer drugs and chemical modulators of anticancer drug effects.

The LETM is presently organized into two sections.

Pharmacology and Toxicology Section.

As part of the LETM, the overall goal of the Pharmacology and Toxicology section is to provide basic information pertaining to the biochemistry, pharmacology, and toxicology of chemicals that are useful in our understanding and improvement of cancer therapy. Personnel in the Pharmacology and Toxicology section utilize an interdisciplinary and collaborative approach to the project areas summarized below.

a) In Vivo Metabolism and Toxicity; Effects of Thiol Modulation

Two classes of chemical agents, 2-chloroethylnitrosoureas and alkylfurans, have organ specific toxicity in vivo that relates to thiol modulation. In vivo administration of buthionine sulfoximine (BSO) depleted rat tissue thiols with the greatest depletion in kidney tissue and bone marrow thiols being the most resistant to thiol depletion. BSO pretreatment increased the renal and hepatic toxicity of MeCCNU in rats and increased the lethality of L-PAM in BDF mice. Liver GSH levels decreased after administration of 2-methylfuran which displayed enhanced toxicity after phenobarbital treatment of rats. Diethylmaleate treatment enhanced toxicity and covalent binding of 2-methylfuran presumably due to GSH depletion. However, GSH depletion by BSO protected against 2-methylfuran toxicity and covalent binding.

DNA alkylation and nephrotoxicity of MeCCNU indicate that it may not be possible to dissociate the antitumor activity of the nitrosoureas from their potential for nephrotoxicity. However, nephrotoxicity appears independent of carbamylating activity of MeCCNU. Hepatic metabolism of MeCCNU by cytochrome P-450

dependent reactions to reactive intermediate(s) of the 2-chloroethyl side chain may contribute to the DNA alkylation and renal toxicity. Metabolic studies with A-(2-chloroethyl) conjugates of cysteine and glutathione indicate a possible role of these metabolic intermediates in nephrotoxicity of chloroethylnitrosoureas.

An animal model that utilizes multidosing has been utilized to examine the mechanism of BCNU-induced pulmonary toxicity. Electron microscopic studies have demonstrated that BCNU causes selective toxic changes in pulmonary alveolar type II cells suggestive of disturbed phospholipid synthesis at doses of BCNU that no other cell type examined was affected. A marked decrease in pulmonary, but not renal, fatty acid synthesis rates was noted after BCNU treatment.

A novel protocol was utilized to evaluate the nephrotoxic potential of tetraplatin, a new platinum analog. The nephrotoxicity of tetraplatin was compared with cisplatin at equimolar doses and found to be less toxic and markedly different. The first indication of neurotoxicity was a severe diuresis and certain kidney function parameters were found to be inappropriate for assessing the onset of nephrotoxicity. Urinary excretion of proteins, glucose and enzymes was the most sensitive indicator for the onset of neurotoxicity. Differences between the toxicity of tetraplatin and cisplatin include hematologic disorders and gastrointestinal toxicity.

b) In Vitro Nephrotoxicity Model with Renal Slices

An in vitro model using renal cortical slices has been developed to evaluate acutely-acting proximal tubular nephrotoxicants. Fischer 344 rat kidney slices provided a useful model for mechanistic evaluations of several classes of nephrotoxicants. Data obtained with in vitro slice exposure appear to correlate well with in vivo nephrotoxicity; quantitative comparisons between different nephrotoxicants do not appear justified and the model does not appear to be useful for routine screening of unknown compounds. Mechanistic evaluations for certain classes of nephrotoxicants appear to be a very useful application of this in vitro model.

c) Human Lung Carcinoma Cell Line Model Systems

Biochemical characterization of human lung carcinoma cell lines (HLCCL) has continued to provide a basis for target cell-oriented lung cancer therapy. The cell lines NCI-H332 and NCI-H358, derived from pulmonary adenocarcinomas metabolized arachidonic acid (AA) to PGE_2 which was characterized by negative ion chemical ionization GC-MS analysis. The prostaglandin endoperoxide synthetase (PES) activity was also measured by the amount of immunoreactive PGE_2 formed under a variety of incubation conditions. No detectable PGE_2 was formed by the small cell cancer cell lines NCI-H69 and NCI-H128 when incubated in the presence or absence of calcium ionophore A23187. NCI-H358 cells failed to metabolize polyunsaturated fatty acids other than AA. These findings suggest that powerful biochemical tools can be developed to understand important pathophysiological aspects of human lung cancer that may have useful diagnostic applications during cancer therapy. Other studies include the observation that a homogenous human pulmonary large cell carcinoma cell line NCI-H460 is unique from other HLCCL in that PGE_2 synthesis activity is much higher in early passage as compared to that in late passage. These changes are being correlated with ultrastructural changes which may provide additional diagnostic application of clinical value.

d) Cell-Free In Vitro Formation of Reactive Intermediates

Prostaglandin endoperoxide synthetase (PES) catalyzed cooxidation was found to occur with mitoxantrone, bromophenol and 2-bromohydroquinone. The human pulmonary adenocarcinoma cell lines, NCI-H322 and NCI-H358, which contain high PES activity are susceptible to mitoxantrone-induced cytotoxicity.

Adriamycin stimulated NADPH-dependent lipid peroxidation several-fold in microsomes but not mitochondria isolated from EMT6 mammary carcinoma cells and B16 solid tumors. In separate experiments, it was found that in the presence of either NADPH or NADH, GSH increased endogenous and adriamycin-enhanced mouse kidney microsomal lipid peroxidation, in contrast to the known inhibitory effect of GSH in other peroxidation systems. These findings indicate much more needs to be understood about lipid peroxidation processes as related to both normal and tumor tissues during anthracycline chemotherapy.

Pathology and Ultrastructural Oncology Section

This Section's research focusses on mechanisms of development and therapy of lung cancer. Studies on the pathogenesis of chemically induced lung cancer in rodents as well as reports on human lung cancers have provided ample evidence that the histopathologic typing of lung cancers and their preneoplastic lesions is insufficient for research in that they lack information on cell type and degree of differentiation of subcellular organelles. Projects of this Section and collaborative efforts with the Pharmacology and Toxicology Section, LETM, as well as other laboratories are therefore based on the concept that response of cells to chemicals including drugs are dictated by specific biochemical reactions of cells in a manner which can be dependent of cell types and differentiation. This approach necessitates the application of electron microscopy for diagnosis of cell type and degree of differentiation at the organelle level to all experiments. Moreover, studies on the effects of chemicals and drugs are generally addressed with the same methodology to provide initial information on target cell type(s) and/or organelles that could then be utilized for the design of more focussed experiments on the underlying biochemical mechanisms.

Although all of our current research is based on information generated in earlier animal experiments, our major emphasis during this report period has been on the characterization and establishment of human lung cancer cell lines (HLCCL) for research on cell type specific chemical/drug interactions. The experience generated through such studies has also enabled us to initiate and design an number of experiments primarily conducted by investigators of the Pharmacology and Toxicology Section, LETM. In summary, we found that:

1. Of seven investigated non-small cell derived HCCL, only two adenocarcinomas NCI-H322 and NCI-H358) exhibited unequivocal well differentiated features of one pulmonary cell type (as assessed by electron microscopy) while all others demonstrated either dual differentiation (e.g., adenosquamous) or poor differentiation which made a diagnosis by cell type questionable. We also found evidence that well differentiated lung tumors undergo pronounced morphological changes during prolonged maintenance in vitro. This exemplifies the need for careful electron microscopic assessment of HCCL before and during their use for biochemical and pharmacological experiments.

2. Similar to in vivo studies in hamsters, the pulmonary carcinogen, diethylnitrosamine (DEN), is preferentially metabolized by HLCCL with features of pulmonary Clara cells in vitro and that such metabolism is inhibitable by inhibitors of cytochrome P-450 enzymes.

3. HLCCL of different cell types metabolize DEN via different enzyme systems, e.g., Clara cells via cytochrome P-450 and alveolar type II cells via prostaglandin endoperoxide synthetase. Cell type specific differences such as this in nitrosamine activation may well be responsible for the known organ and cell specific carcinogenicity of these compounds.

4. Similar to experiments in rats, the pulmonary toxin and candidate anticancer drug, 4-ipomeanol, is preferentially metabolized by an HCCL with characteristics of Clara cells and that such metabolism is mediated by cytochrome P-450 enzyme systems. The selective toxicity of the compound for this cell line suggests to pursue its usefulness as an anticancer drug for pulmonary adenocarcinomas and other solid tumors of similar cell types.

5. The xenobiotic-metabolism enzymes, cytochrome P-450, arylhydrocarbon hydroxylase and ethoxycoumarin O-diethylase activity are selectively present in non-small cell derived HCCL while they are lacking in small cell derived HCCL.

6. Normal alveolar type II cells of rat lung proliferate in vitro until 7 days in culture, retain their typical ultrastructure and phospholipid synthesis and can be identified by immunoperoxidase using an antiserum specifically for rat surfactant apoprotein.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07140-03 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: BCNU - Induced Pulmonary Fibrosis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	A. C. Smith	Staff Fellow	LETM, NCI
Others:	H. M. Schuller	Vet. Med. Officer	LETM, NCI
	M. R. Boyd	Associate Director	DTP, NCI
	J. B. McMahon	Cancer Expert	LETM, NCI
	D. J. Reed	IPA	LETM, NCI
	R. A. Gram	Bio. Lab. Aid	LETM, NCI

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pharmacology and Toxicology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	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 potent alkylating antitumor agent, causes a severe pulmonary injury in patients treated with high doses of the drug. We have described a multi-dosing animal model to study the mechanism of BCNU-induced pulmonary toxicity. After 2 doses of BCNU (10 mg/kg cumulative dose), electron microscopic studies have shown changes in pulmonary Type II cells which are associated with cell toxicity and disturbed surfactant synthesis. Alveolar Type II cells are responsible for surfactant production and have a very active lipid biosynthesis system. Therefore, fatty acid synthesis was measured as a marker for pulmonary Type II cell injury after BCNU administration. Single doses of BCNU caused a dose- and time-dependent decrease in fatty acid synthesis rates measured in lung tissue. A maximum of 48% of ^{14}C-acetate incorporation into fatty acids occurred 24 hours after a dose of 120 mg BCNU/kg. This depression in fatty acid synthesis lasted up to 48 hours after a single dose of BCNU. The multi-dosing model for BCNU-induced pulmonary fibrosis also resulted in a marked alteration of pulmonary fatty acid synthesis rates. After 6 doses of BCNU (cumulative dose 30 mg/kg) fatty acid synthesis was depressed by 64%. This inhibition was organ-specific, renal rates of ^{14}C-acetate incorporation into fatty acids did not change after 6 doses of BCNU when maximal changes in lung tissue occurred. This correlated with an absence of kidney damage measured by organic ion accumulation or lustology. Rates of fatty acid synthesis appear to be a good biochemical marker for pulmonary alveolar Type II cell injury produced by BCNU. Freshly isolated rat pulmonary Type II cells are sensitive to BCNU in vitro. Incubation of confluent cultures of Type II cells with BCNU resulted in a depression of cellular glutathione. These alterations of the Type II cells antioxidant defense mechanism occurred with evidence of cell injury, but no cell death. This indicates, perhaps an additional oxidant challenge is necessary to produce overt cell death.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea, Carmustine) is a very potent anti-tumor xenobiotic used in the treatment of a wide variety of human neoplasms. BCNU therapy is associated with numerous drug-related side effects, the one most recently described is the development of a life-threatening pulmonary fibrosis after BCNU treatment. Our laboratory has developed an animal model to study BCNU-induced pulmonary injury in F344 rats. Animals treated with 5 mg BCNU/kg once a week for 6 weeks develop a progressive pulmonary fibrosis which is histologically similar to the lung disease which develops in patients treated with high doses of BCNU. Electron microscopic studies aimed at following the progression of lung injury caused by BCNU treatment have demonstrated that relatively low doses of BCNU cause selective toxic changes in pulmonary alveolar Type II cells suggestive of disturbed surfactant synthesis. No other cell type examined (endothelial, Clara, alveolar Type I cells) was effected by small doses of BCNU. This led us to postulate that alveolar Type II cells may be the target cell population for BCNU-induced lung injury.

Pulmonary Type II cells are located in the alveolar wall and are believed to be stem cells for replacement of alveolar Type I cells which line the alveolus. Type II cells are also thought to be the major site for the production of pulmonary surfactant. Surfactant, a phospholipid- rich material, which acts to coat the alveolar cell surface thus lowering the surface tension and preventing the collapse of the alveolus during respiration. Therefore, Type II cells have a very active lipid biosynthesis system and Type II cell toxins have a marked effect on total lung lipid biosynthesis. Studies were initiated to explore the effect of BCNU on Type II cell function in vivo by measuring pulmonary fatty acid synthesis rates.

Other studies in our laboratory have been concerned with the mechanism of BCNU-induced lung damage. Recent work suggested that the effect of BCNU on the pulmonary glutathione redox system, an important cellular antioxidant defense mechanism, may result in the onset of pulmonary injury. We have demonstrated in vivo that there is a temporal relationship between the alteration of the pulmonary glutathione system and the onset of severe lung damage. Since Type II cells appear to be the target cell for BCNU-induced lung injury, studies were initiated to explore the role of alterations in the antioxidant defense mechanism in the toxicity of BCNU on freshly isolated rat pulmonary Type II cells.

Methods Employed:

A. Effect of BCNU on fatty acid synthesis

Male Fisher 344 rats (8 weeks old) were treated with BCNU intraperitoneally. For multi-dosing studies, rats were injected with 5 mg BCNU/kg dissolved in 10% ethanol. Higher concentrations of BCNU were needed for single dose studies and therefore BCNU was dissolved in sesame oil.

After BCNU administration, rats were sacrificed by exsanguination. Lungs were removed, trimmed, lobes were separated and rinsed in buffer. Slices were made by cutting the lobe with a series of razor blades separated by washers. Slices

were very uniform and approximately 0.4 - 0.5 mm thick. Each incubation contained 100 mg lung tissue (about 6-8 slices) in 3 ml of PFMR-4 media. Slices were preincubated for 15 minutes at 37°C under 95% O_2:5% CO_2. Acetate (5 μCi sodium [μ-^{14}C] acetate in 15 μmoles of cold acetate) was added and samples were incubated for an additional 60 minutes. The reaction was terminated when the lung slices were removed and homogenized in chloroform:methanol (2:1) to extract lung lipids. The organic layer was washed 2 times with 1.0 ml of 0.1 M phosphate buffer (pH 7.4) saturated with chloroform:methanol (2:1). The solvent was removed under nitrogen and lipids were resuspended in 1.5 ml chloroform. Radioactivity was determined in duplicate aliquots.

B. Effects of BCNU on isolated rat pulmonary Type II cells in vitro

F344 rat alveolar Type II cells were isolated by the method described in this annual report. Confluent 4 day Type II cell cultures containing approximately 1.8 x 10^6 cells were suspended in F12 media containing no serum and exposed to 50 μ M BCNU for 4 hours at 37°C under 5% CO_2. Total glutathione levels were determined by high pressure liquid chromatography according to the method described by Reed and coworkers (Anal. Biochem. (1980) 106:55). Reduced glutathione was used to establish a standard curve and γ-glutamylglutamate was used as an internal standard.

Results:

A. In vivo effect of BCNU on pulmonary fatty acid synthesis.

Single doses of BCNU had a profound effect on pulmonary rates of fatty acid synthesis measured in lung slices. There was a rapid 25% decrease in fatty acid synthesis in lung tissue 4 hours after BCNU administration. This inhibition of fatty acid synthesis remained up to 48 hours after a single dose of BCNU. Increased doses of BCNU resulted in a larger amount of fatty acid synthesis inhibition. A single dose of 90 mg BCNU/kg or 120 mg BCNU/kg inhibited, respectively, 36% and 48% of total lung ^{14}C-acetate incorporation into fatty acids 24 hours after BCNU. The multi-dosing model for BCNU-induced pulmonary fibrosis also resulted in a marked depression of pulmonary rates of fatty acid synthesis. Three doses of BCNU (cumulative dose 15 mg/kg) resulted in a 40% depression of ^{14}C-acetate incorporation into lung phospholipids. Six doses of BCNU (cumulative dose 30 mg/kg) caused fatty acid synthesis rates to fall to 52% of control levels. Again, this inhibition of ^{14}C-acetate incorporation in lung lipids by BCNU was persistant, lasting up to 48 hours after a dose of BCNU.

The effects of BCNU on fatty acid synthesis rates were specific for lung tissue. After 6 doses of BCNU (cumulative dose 30 mg/kg) which resulted in a 50% inhibition of ^{14}C-acetate incorporation into lung lipids did not result in any alteration of fatty acid synthesis in kidney tissue. This was also no evidence of kidney damage after BCNU administration when measured biochemically by organic ion accumulation or morphologically. This information demonstrates that changes in fatty acid synthesis rates after BCNU are specific for the target organ, the lung, and are presumably due to Type II cell injury caused by BCNU administration.

B. The effect of BCNU on isolated pulmonary Type II cells in vitro.

Preliminary experiments have been conducted to establish the sensitivity of freshly isolated rat pulmonary Type II cells to BCNU treatment in vitro. Of particular interest is to establish the role of the alteration of the antioxidant defense mechanism in BCNU-induced Type II cell injury. BCNU causes a time- and dose-dependent decrease in cellular glutathione levels. Four hours after exposure of Type II cells to BCNU, approximately 58% of total glutathione was depleted due to a marked inhibition of cellular glutathione reductase by BCNU. With this marked alteration of the cellular antioxidant defense mechanism, there was an increase in cell injury measured by lactate dehydrogenase leakage, but no indication of cell death measured morphologically. These studies indicate that pulmonary Type II cells are sensitive to BCNU toxicity in vitro as well as in vivo.

Significance:

BCNU therapy often causes a fatal pulmonary fibrosis in patients treated with high doses of the drug. This research project is aimed at elucidating the mechanism of BCNU-induced pulmonary injury. We have shown by electron microscopy that pulmonary Type II cells are the only cell type affected by low doses of BCNU and that this morphological evidence of injury correlates with biochemical changes (i.e., fatty acid synthesis rates) associated with pulmonary Type II cells. Fatty acid synthesis rates is a sensitive biochemical marker for pulmonary Type II cell injury caused by BCNU can be used to monitor and quantitate lung damage in vivo. We have also shown that freshly isolated rat pulmonary Type II cells are sensitive to BCNU-induced damage in vitro. Preliminary results indicate that after exposure to low concentrations of BCNU there are marked alterations in the glutathione redox systems and these changes occur with apparent cell toxicity but no cell death. These studies suggest that perhaps an additional oxidant challenge is necessary for BCNU to cause overt cell death.

Proposed Course:

Current studies are aimed at specifically addressing the effect of BCNU on the glutathione redox system in freshly isolated rat pulmonary Type II cells. There are several questions to be answered pertaining to the role BCNU-induced alterations of the glutathione redox status in Type II cells with oxidant damage. These studies will indicate perhaps the type and extent of oxidant challenge necessary for cell death and also may help to elucidate the mechanism of BCNUinduced pulmonary fibrosis in vivo.

Publications:

1. A.C. Smith and M.R. Boyd (1984) Preferential effects of 1,3-bis(2-chloroethyl)-1-nitrosourea on pulmonary glutathione reductase and GSH/GSSG ratio: Possible implications for lung toxicity. J. Pharmacol. Exp. Ther. 229, 658-663.

2. H.M. Schuller, A.C. Smith, M. Gregg and M.R. Boyd (1985) Pathogenesis of pulmonary fibrosis induced in rats with the anticancer drug 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU). Exp. Lung Res. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07160-02 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

In vitro Model for Evaluating Nephrotoxicity

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J. H. Smith	PRAT Staff Fellow	NIGMS
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COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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 spece provided.)*

An in vitro model using renal cortical slices has been developed to evaluate acutely-acting proximal tubular nephrotoxicants. The toxicity of several classical nephrotoxicants (including $HgCl_2$, $K_2Cr_2O_7$, CCl_4, hexachlorobutadiene, cephaloridine, gentamicin and 4-ipomeanol) has been evaluated in this sytem using Fischer 344 rat kidney. Conditions have been established where several physiologic parameters can be monitored after a single incubation of 100 mg of renal cortical slices. Although the data for in vitro slice exposure appear to correlate well with in vivo nephrotoxicity, quantitative comparisons between different nephrotoxicants are probably inappropriate. Thus, this model will not be useful for routine screening of unknown compounds. Nevertheless, there are similarities in the characteristics of nephrotoxicity observed in vitro and those reported in vivo, suggesting the model may be useful for mechanistic evaluations of certain classes of nephrotoxicants. Preliminary data suggest this model may be of particular use in evaluating nephrotoxicity of platinum anticancer drugs.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives:

Although the kidneys are susceptible to toxicity by a variety of xenobiotics, and especially anticancer drugs, relatively little is known regarding mechanisms of nephrotoxicity. Renal cortical slices perpared from laboratory animals have been used to evaluate renal toxicity, in combination with urinary, serologic and histologic evaluations. In acute toxicity evaluation, renal cortical slices usually are prepared 24 hr or longer after nephrotoxicant administration and several physiologic parameters are determined. Recently, the mechanism of chloroform nephrotoxicity was determined by monitoring organic ion accumulation after in vitro exposure of renal cortical slices from mice, rabbits and rats to chloroform. This in vitro model allowed an evaluation of the relationship between in situ renal metabolism of a compound and the subsequent nephrotoxicity. The purpose of this investigation was to determine whether renal cortical slices could be used to evaluate other acutely-acting proximal tubular nephrotoxicants. Because decreases in organic ion accumulation do not necessarily reflect impaired proximal tubular function due to toxicity, particularly after in vitro exposure to a chemical, other measures of proximal tubular function and cell integrity were incorporated into the procedure.

Methods Employed:

Approximately 100 mg of thin renal cortical slices are prepared from freshly isolated Fischer 344 rat kidneys. Slices are placed in 2 ml of a phosphate-buffered medium on ice until all slices are prepared for the days' incubations, usually a period of 1 to 1 1/2 hr. This brief holding period serves to rinse the freshly sliced tissue. The slices are transferred to 25 ml Erlenmeyer flasks containing 4 ml of the preincubation media and various concentrations of the nephrotoxicant to be evaluated. Flasks are gassed with 100% O_2 for 5 min, stoppered, and preincubated at 37°C for 2 hr in an oscillating water bath. After this preincubation, slices are rinsed in fresh media lacking the nephrotoxicant, blotted gently, and transferred to fresh media for a subsequent incubation at 25°C. This incubation is performed to assess proximal tubular cell function by monitoring gluconeogenesis, organic ion accumulation and tissue glutathione concentrations. The preincubation and incubation media are monitored for renal marker enzymes, which have been assessed in the urine clinically as a non-invasive monitor of nephrotoxicity. Enzyme activities originating from brush border [alkaline phosphatase (AP), γ-glutamyl transpeptidase (γGT), maltase], cytosol [lactate dehydrogenase (LDH)] and lysosomes [N-acetyl- βglucosaminidase (NAG)] are determined. Additionally, preincubation media is monitored for the presence of malondialdehyde (MDA) as an indication of lipid peroxidation.

Major Findings:

Conditions have been established where several physiologic parameters can be monitored after a single incubation of renal cortical slices (~100 mg); previous investigations have assessed each of these individually. These parameters are relevant to tests used for assessment of nephrotoxicity after in vivo administration of a nephrotoxicant.

Compounds representing several classes of nephrotoxicants have been tested in this in vitro slice model. In vitro concentrations were chosen which were equivalent to or less than the total number of moles administered in vivo to produce nephrotoxicity. The compounds evaluated include $HgCl_2$, $K_2Cr_2O_7$, CCl_4, hexachlorobutadiene (HCBD), cephaloridine, gentamicin, and 4-ipomeanol. 4-Ipomeanol is not nephrotoxic to rats and was, therefore, used as a negative control. The concentration of 4-ipomeanol was based on the LD_{50} of this compound.

Measurements of proximal tubular cell function appeared to be most sensitive in detecting cell toxicity. The concentration-response curves for organic ion accumulation and for gluconeogenesis were similar. On initial observation, the data from in vitro slice exposure appeared to correlate well with in vivo nephrotoxicity of these compounds. Upon more critical analyses of these data, however, it appeared that quantitative comparisons between different nephrotoxicants are probably inappropriate. Consistent with in vivo nephrotoxicity, the metals $HgCl_2$ and $K_2Cr_2O_7$ were the most nephrotoxic. The solvents CCl_4 and HCBD followed in toxicity. At the higher concentrations, neither solvent was completely soluble in the aqueous buffer. Therefore, it was not clear whether the "toxicity" observed in this in vitro model represented a toxic mechanism similar to that occurring in vivo, or whether this "in vitro" toxicity represented nonspecific solvent effects on membrane integrity. Relatively higher concentrations of cephaloridine and gentamicin were required to produce toxicity in this model This was consistent with these drugs where nephrotoxicity is delayed or requires multiple doses, and where toxicity appears to be correlated directly with renal accumulation of the drug. 4-Ipomeanol gave no indication of nephrotoxicity on any of the parameters measured in this model in the concentration range approximating the LD_{50} (1 mM in this system).

Release of the cytosolic enzyme, LDH, is used routinely for assessing damage in cell cultures and freshly isolated cells. The relative responses of LDH release for CCl_4, HCBD, cephaloridine and gentamicin were similar to slice function data. LDH release was not a good indicator of cell toxicity after exposure to $HgCl_2$ and $K_2Cr_2O_7$. Release of NAG was similar to LDH except $HgCl_2$ dramatically increased NAG release as well. In general, release of brush border enzymes was a very poor indicator of cell damage in this model. The largest increase of AP, γGT and maltase release was observed only after exposure to the solvents CCl_4 and HCBD. Thus, this elevation may be related to the physical condition of the cells and membrane integrity as a result of the solvent, detergent-like, effects. In all cases, it was preferable to monitor for enzyme activity in the incubation medium rather than the preincubation medium. This was because some compounds, such as $HgCl_2$, directly inhibited the activity of several enzymes.

There was evidence of lipid peroxidation, monitored by MDA formation, in slices preincubated with $HgCl_2$ and cephaloridine. These data are consistent with other investigations reporting lipid peroxidation by these compounds both in vivo and in vitro.

Significance to Biomedical Research and the Program of the Institute:

Nephrotoxicity is an undesirable side-effect of several commonly used anti-cancer agents. Traditionally, nephrotoxicity has been monitored and alterna-

tive dosage regimens have been employed to minimize the potential for toxicity. Unfortunately, relatively little is known regarding the actual mechanism of nephrotoxicity of these compounds. Such knowledge may lead to the development of second generation drugs with chemotherapeutic efficacy which lack the nephrotoxicity, or, alternatively, to the development of methods to avoid the nephrotoxic effects of currently used drugs. An in vitro model model offers the opportunity to assess directly the renal metabolism and toxicity of drugs and chemicals independent of variables such as pharmacokinetics or toxicity to other target organs. In addition, the development of an in vitro model for nephrotoxicity can provide a rapid, inexpensive in vitro target organ screen for a variety of chemicals and drugs. Thus far, this model appears to correlate with characteristics of nephrotoxicity observed in vivo. Since the tissue is isolated rapidly from the animal, this should decrease the potential for dramatic metabolic alterations and other similar artifacts that may be encountered when using isolated cells or cell culture techniques. These advantages may facilitate investigations of species, strain and sex differences in nephrotoxicity and, ultimately, may provide information on more appropriate animal models for toxicological evaluations. Specifically, the model offers the opportunity to utilize human renal tissue for comparative purposes.

Proposed Course:

Evaluation of a variety of classical nephrotoxicants has indicated the benefits and limitations of this in vitro slice model. Currently, this in vitro slice model is being used to evaluate the relative nephrotoxicity of a group of platinum anticancer agents including cisplatin, CBDCA, CHIP and tetraplatin (NSC 119875, 241240, 256927 and 363812, respectively). These data obtained using Fischer 344 rat renal cortical slices will be compared to the in vivo nephrotoxicity of these drugs (see Annual Report Z01 CM 07169-01 LETM). Attempts are being made to obtain human renal tissue within 24 hr after death for similar in vitro nephrotoxicity assessments. The goal of this investigation will then be to use this model for mechanistic evaluation of the nephrotoxicity and of those agents which may reduce nephrotoxicity of platinum anticancer agents. Additionally, collaborative efforts are being made to evaluate histologically the condition of the renal cortical slices subsequent to the preincubation period. This evaluation will address the question of whether cell specific regions of the nephron are damaged in vitro similar to damage occurring in vivo.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07161-02 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: In Vivo Studies on the Toxicity of Alkylfurans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	V. Ravindranath	Visiting Fellow	LETM, NCI
Others:	M. R. Boyd	Associate Director	DTP, NCI

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pharmacology and Toxicology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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 standerd unreduced type. Do not exceed the space provided.)*

The mechanisms involved in the metabolic activation and toxicity of 2-methylfuran (2-MF), a naturally occurring cytotoxic furan found in cigarette smoke and coffee are being investigated.

Following administration of 2-[(^{14}C)methyl]furan to rats, extensive covalent binding of label to macromolecules in liver, lungs and kidney was observed. Smaller amounts were bound to tissues with little or no known mixed function oxidase activity. Maximal covalent binding to both protein and DNA was observed in the liver, the target organ where toxicity is manifested. Liver GSH levels decreased by a third, half an hour after administration of 2-MF indicating the formation of electrophilic metabolites. Pretreatment with various inhibitors and inducers of metabolism showed that phenobarbital potentiated toxicity of 2-MF and was followed by increased urinary excretion of label, while 3-methylcholanthrene or piperonyl-butoxide did not markedly alter the toxicity of 2-MF. Pretreatment with buthionine sulfoximine (BSO), a GSH depletor and a chemosensitizing agent, while decreasing covalent binding, also decreased toxicity, whereas diethylmaleate, also a GSH depletor, increased both covalent binding and toxicity of 2-MF. BSO, which depletes GSH levels by inhibiting cysteine synthetase is known to enhance cysteine levels in tissues. Thus, BSO probably decreases covalent binding by trapping the reactive intermediate as the stable cysteine conjugate. Pretreatment with L-2-oxo thiazoline 4-carboxylate (OTZ), promoter of GSH biosyntheisi potentiated hepatotoxicity and increased covalent binding to liver proteins, although coadministration of OTZ and BSO prior to an i.p. dose of 2-MF, decreased the covalent binding to liver proteins and the serum GTP levels compared to that of rats that received 2-MF alone. The GSH conjugate of the reactive metabolite of 2-MF is unstable, which may explain the potentiation of hepatoxicity of 2-MF by OTZ.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

The toxic alkylfurans, namely, 3-methylfuran (3-MF), a major constituent of atmospheric smog, and 2-methylfuran (2-MF), a natural product present in many foods, cigarette smoke and coffee, are activated by microsomal mono-oxygenase to reactive metabolites that bind covalently to microsomal proteins. Using semicarbazide as a trapping agent, acetylacrolein (AA) and metylbutenedial (MBD) were isolated as the products of microsomal oxidation of 2-MF and 3-MF, respectively. A comparison of the covalent binding of ^{3}H-3-MF and the amounts of methylbutenedial disemicarbazone produced in microsomal incubation in the presence and absence of NADPH and semicarbazide revealed an inverse relationship. Semicarbazide strongly inhibited the NADPH dependent covalent binding of the alkylfurans presumably by trapping the reactive aldehyde before it could react with tissue macromolecules (Science, in press).

Studies were designed to examine the metabolism and toxicity of 2-MF in vivo in rats and to correlate the toxicity with the observed covalent binding using time-course and dose-response relationship. The effect of various inducers and inhibitors of cytochrome P-450 metabolism on the toxicity and covalent binding of 2-MF was also to be examined. In view of the instability in vitro of the glutathione conjugate of the reactive metabolite of 2-MF, namely acetylacrolein, the effects of various inducers and depletors of glutathione on the toxicity of 2-MF was undertaken.

Methods Employed:

An original synthesis of 2-([^{14}C]methyl)furan was first developed using 2-furyl lithium and ([^{14}C]methyl) iodide. Male Sprague Dawley rats were injected with varying doses of ^{14}C-2-MF intraperitonially and were sacrificed 0.5 to 24 hr after dosage. Liver, lung, kidney, blood and muscle were monitored for total radioactivity, radioactivity extractable with methanol and radioactivity covalently bound to both protein and DNA. Glutathione levels were determined in liver, lungs and kidneys. Serum glutamic pyruvic transaminase levels were determined. Samples of liver, lung and kidney were fixed in formalin, sectioned and stained with hematoxylin and eosin and examined by light microscopy. Urine was collected after dosage with ^{14}C-2-MF for isolation of mercapturic acid conjugates.

Major Findings:

When ^{14}C-2-MF was administered in vivo to rats, large amounts of radioactivity were covalently bound to macromolecules in the liver, lungs and kidney and smaller amounts were bound to tissues with little or no known amounts of mixed function oxidase activity. Maximal covalent binding to both protein and DNA was observed in the liver. Covalent binding to liver proteins was five times greater than kidney per mg protein. Damage to liver was observable biochemically by a rise in serum glutamic pyruvic transaminase levels and histopathologically by centrilobular necrosis of liver. Liver GSH levels decreased by 33% half an hour after a dose of 2-MF indicating the formation of reactive electrophilic metabolites and the role of endogenous GSH as a protecting agent.

Pretreatment with phenobarbital enhanced the toxicity of 2-MF. Over a 12 hr period there was an increased urinary excretion of 2-MF metabolites in rats pretreated with phenobarbital. Pretreatment of rats with phenobarbital also greatly enhanced the level of covalent binding of the label to proteins in liver, lungs and kidney. Covalent binding to liver proteins and DNA increased two-fold with phenobarbital pretreatment. Pretreatment with N-octylimidazole (Wilkinson et al., 1976), a potent inhibitor of cytochrome P-450 markedly decreased the level of covalent binding of the label from ^{14}C-2-MF to proteins and DNA in liver, lungs and kidney. N-octylimidazole pretreatment completely ameliorated any increase in SGPT levels caused by 2-MF, pretreatment with N-octylimidazole completely prevented the hepatotoxicity caused by 2-MF treatment. SGPT levels of rats treated with 3-methylcholanthrene or piperonyl butoxide prior to 2-MF administration were not different from rats that received 2-MF alone.

Prior dosing with diethylmaleate increased covalent binding of 2-MF metabolites in liver, lungs and kidney. However, pretreatment with buthionine sulfoximine (BSO), which depletes GSH by inhibiting GSH synthesis, decreased both toxicity and covalent binding of 2-MF metabolites in anamoly to the effects observed with diethylmaleate pretreatment. BSO is known to inhibit cysteine synthetase and increase the concentration of cysteine. In vitro, cysteine is a better inhibitor of covalent binding of 2-MF metabolites than GSH. Cysteine can form a cyclic conjugate with acetyacrolein which is more stable than the GSH-acetylacrolein conjugate. Thus, it seems that BSO inhibits covalent binding by trapping the reactive metabolites as cysteine conjugates. The effects of BSO pretreatment on 2-MF covalent binding is marked in the liver where there is a rapid turnover of GSH. Pretreatment with oxythiazolidine, an inducer of GSH, increased both toxicity and covalent binding of 2-MF metabolites in liver. But a combination of oxythiazolidine and BSO, which is known to nullify the effects of oxythiazolidine, decreased both toxicity and covalent binding significantly. The possible reason for the increased covalent binding observed with oxythiazolidine could be that the increased GSH levels prevent the suicidal destruction of cytochrome P-450 by the reactive metabolite of 2-MF by forming the GSH-conjugate. But, the GSH-acetylacrolein conjugate is known to be unstable, hence, the acetylacrolein released from the GSH conjugate could react with other proteins. Further, the acetylacrolein-GSH conjugate can react with proteins by the virtue of its free aldehyde moiety. Thus, there is increased metabolism of 2-MF in the presence of increased levels of GSH, but due to the instability of the GSH conjugate it cannot effectively prevent the covalent binding of the reactive metabolite.

Significance to Biomedical Research and the Program of the Institute:

Identification of α - β unsaturated aldehydes as the major reactive metabolites of alkylfurans leads to a new class of reactive metabolites which can react specifically with tissue macromolecules in a number of ways. Unsaturated aldehydes can react with both proteins and DNA either via Michael addition across the activated double bond or nucleophilic addition to the aldehyde. In view of the bifunctional reactive sites interstrand crosslinks and protein and DNA crosslinks are possible, which may be important in view of the potential carcinogenicity and/or cytotoxicity effects of the toxic furans. The decreased

covalent binding of 2-MF metabolites seen after pretreatment with BSO is also of importance in view of the role of BSO as a radiosensitizing agent in cancer chemotherapy particularly with alkylating agents.

Proposed Course:

The major objectives of this project are nearly complete and the manuscripts are in preparation.

Publications:

1. Boyd, M.R., Ravindranath, V., Burka, L.T., Dutcher, J.S., Franklin, R.B., Statham, C.N., Haschek, W.M., Hakkinen, P.J., Morse, C.C. and Witschi, H.P.: Drug metabolizing enzyme systems and their relationship to toxic mechanisms. In: Proceedings of New Approaches in Toxicity Testing and their Application to Human Risk Assessment, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07162-02 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Role of Arachidonic Acid Metabolism in Human Lung Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. C. Hubbard	Cancer Expert	LETM, NCI
Others:	E. G. Mimnaugh	Chemist	LETM, NCI
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	H. M. Schuller	Vet. Med. Officer	LETM, NCI
	S. S. Lau	Sr. Staff Fellow	LETM, NCI
	M. R. Boyd	Associate Director	DTP, NCI
	M. G. McMenamin	Chemist	LETM, NCI
	K. E. Greene	Bio. Lab. Tech.	LETM, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.7	0.3

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 metabolism of arachidonic acid (AA) has been determined in four lines. The cell lines NCI-H69 and NCI-H128 are derived from small cell carcinomas of the lung (SCCL), and the cell lines NCI-H322 and NCI-H358 are derived from pulmonary adenocarcinomas. AA did not undergo metabolism when incubated with suspensions of NCI-H69 and NCI-H128 cells. Significant metabolism did occur when AA was incubated with monolayers of unstimulated NCI-H322 and NCI-H358 cells. A single metabolite of AA isolated from these cells was identified as prostaglandin E_2 (PGE_2) via combined gas chromatography-mass spectrometry (GC-MS). Additional details of arachidonate metabolism in monolayers of these cells are contained in a separate report (see No. 07173-01). The preferential synthesis of prostaglandins by certain types of human lung carcinomas may have direct clinical implications. Elevated levels of production of selected prostanoids in patients could be indicative of the presence of certain cell types of lung carcinomas. The selective synthesis of prostanoids by certain human lung carcinomas may also confer significant capabilities for P-450 monooxygenase-independent metabolism of antitumor agents and other xenobiotics. The possibility that prostaglandin synthesis may be a unique feature of certain types of human lung carcinomas and the role of the prostaglandin endoperoxide synthase (PES) system in the metabolism of antitumor agents and other xenobiotics are under investigation.

Methods Employed:

GC/MS analysis: PGE_2 was identified as the major metabolite of arachidonic acid synthesized by the NCI-H322 and NCI-H358 cells via negative ion chemical ionization (NICI) GC-MS analysis. Aliquots of 200 µl of the HBSS removed after incubation with the NCI-H358 and NCI-H322 cells were placed in a vial containing ten nanograms of 3,3,4,4-tetradeutero (d_4) prostaglandin E_2. The pH of the HBSS was titrated to pH 3.2 followed by extraction with diethyl ether. The ether extract was transferred to a 1.0 ml conical vial and derivatized to the methyloxime-pentafluorobenzyl-bistrimethylsilyl ether (MO-PFBE-TMS) derivative. PGE_2 was identified via monitoring the characteristic fragment ions m/z 524 (unlabeled PGE_2) and m/z 528 (d_4-PGE_2), eluting at the retention time of the MO-PFBE-TMS derivative of PGE_2 and the distribution of the ion current profiles and total ion current into syn- and anti-isomers characteristic of PGE_2. Salient features of employing NICI GC-MS analysis are presented below (see Proposed Course of Studies).

Characterization of the PES system: Characterization of the PES system requires frequent sampling of the incubation medium for determination of the quantities of PES products formed. In order to eliminate perturbations in temperature and gas equilibrium as a variable in these studies, we are presently adapting our procedures for the proliferation of different human lung carcinoma cells attached to microcarriers.

In addition to adaptations of our culture methods for the proliferation of human lung carcinoma cells, we have investigated the fatty acid cyclooxygenase metabolism of four polyunsaturated fatty acids in monolayers of NCI-H358 cells. These four fatty acids (linoleic, eicosatrienoic, eicosapentaenoic and docosahexaenoic acids) are significant lipid constituents of the culture medium in which the lung carcinoma cells are maintained in vitro and could contribute to the PES activity of human lung carcinoma cells.

Major Findings:

Studies of the metabolism of linoleic, eicosapentaenoic and docosahexaenoic acids in monolayers of NCI-H358 cells indicate that these polyunsaturated fatty acids are not metabolized by the PES system in these cells. Eicosatrienoic acid undergoes oxygenation in the presence of the NCI-H358 cells, presumably to PGE_1. The quantiative relationship between PGE_1 and PGE_2 synthesis from endogenous fatty acid precursors is under investigation. The adaptation of our cell culture procedures have proven successful in the proliferation of the NCI-H358 cell line attached to microcarriers.

Proposed Course:

The future course of these studies can be subdivided into two major areas. First, the kinetics of PES metabolism of arachidonic acid and other polyunsaturated fatty acids will be determined in cell lines NCI-H322 and NCI-H358. These studies will allow us to determine total PES activity of these human lung carcinoma cells and the portion of PES activity that is represented by PGE_2 production in the different cell lines. These studies will require maximizing our yield of cells proliferated attached to microcarriers. Second,

the synthesis of PGE_2 and other eicosanoids in primary cultures of human lung carcinomas and prostanoid production in selected human lung cancer patients will be determined. An essential component of the latter proposal will include the classification of human lung carcinomas upon the basis of ultrastructural features. These latter studies will allow us to determine whether prostaglandin synthesis is a unique characteristic of certain types of human lung carcinomas.

In order to determine the role of PGE_2 and related eicosanoids in the pathophysiology of human lung cancer, it is essential to have the analytical capabilities for the identification and quantitation of prostaglandins and related eicosanoids. These compounds are normally present in body fluids in subnanogram quantities. Three methods for quantitation of prostaglandins and related eicosanoids have been developed. These are: 1) bioassay, 2) radioimmunoassay (RIA) and 3) combined gas chromatography-mass spectrometry (GCMS). Of these three methods of quantitation of these compounds, GC-MS analysis is the most selective. When negative ion chemical ionization of electron-capture derivatives of prostaglandins and related eicosanoids is employed, mass spectrometry is also the most sensitive method for their quantitation with amounts of less than 100 femtograms being reliably quantitated. Thus, mass spectrometry is recognized as the analytical method with which other methods of quantitation are compared.

Four quadrupole mass spectrometer systems with a capillary gas chromatograph interface and operable in the negative ion detection mode were evaluated for sensitivity in the detection and quantitation of electron-capture derivatives of prostanoids. After extensive evaluation of each of the four instruments for sensitivity in the measurement of prostaglandin standards, it was determined that the Finnigan MAT 4610B system was 50-200 times more sensitive than the other instruments at the time of the evaluations. Thus, a recommendation for purchase of the Finnigan MAT 4610B system was made. This instrument is presently on order from Finnigan MAT (Requisition No. 196225; Contract No. 263-85 C-0089) with an anticipated shipment date from the manufacturer of May 31, 1985.

The GC/MS system will be employed for quantitation of prostanoids synthesized by human lung carcinoma cells and for the assessment of prostanoid production in selected human lung cancer patients. The methods for quantitation of PGE_2, other primary prostaglandins and related eicosanoids are similar to those described above (see Methods Employed).

Because of the rapid metabolism of the prostaglandins and related eicosanoids and their formation in cellular elements ex vivo, direct measurement of eicosanoids in either plasma or serum does not provide a reliable index of the production of prostanoids in vivo. For example, it has been shown that the most reliable index of PGE_2 production in vivo is via measurement of either a circulating metabolite or a urinary metabolite of PGE_2. PGE_2 is transformed by 15-dehydrogenation and Δ^{13}-reduction to 15-keto-13,14-dihydro-PGE (15K-H_2-PGE_2). Further degradation of 15K-H_2-PGE_2 via two beta-oxidative steps and omega-oxidation yields (-)-7α-hydroxy-5,11-diketotetranoprostane-1,16-dioic acid, a compound more frequently referred to as PGE-M. PGE-M is the most abundant characteristic metabolite of PGE_2 excreted in urine of several species including humans. We propose to employ the excretion rate of PGE-M as an index

of PGE_2 production in patients with lung carcinomas. Similar approaches of analysis will be employed for the assessment of the in vivo synthesis of other prostanoids if appropriate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07163-02 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Xenobiotic Metabolism by Prostaglandin Endoperoxide Synthetase (PES)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. S. Lau	Sr. Staff Fellow	LETM, NCI
Others:	J. B. McMahon	Cancer Expert	LETM, NCI
	M. R. Boyd	Associate Director	DTP, NCI
	M. G. McMenamin	Biologist	LETM, NCI
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COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.6	0.3

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 site of toxicity of a number of xenobiotics and antitumor agents requiring metabolic activation is distributed in tissues containing very little P-450 monooxygenase activity. Because of the ubiquitous distribution of the prostaglandin endoperoxide synthase (PES) system and the ability of this system to catalyze the cooxidation of xenobiotics to carcinogenic, mutagenic or other reactive species, it has been implicated as playing a significant role in the metabolism of xenobiotics. We have initiated studies on the metabolism of a variety of compounds by ram seminal vesicle (RSV) microsomes (a rich source of PES). These compounds include: a) anticancer agents, such as NMF, cytoxan, PCNU, AZQ, caracemide and mitoxantrone; b) a pulmonary toxin, ipomeanol; and c) nephrotoxins such as o-bromophenol and 2-bromohydroquinone. During prostaglandin biosynthesis, mitoxantrone, o-bromophenol and 2-bromohydroquinone were cooxidized to reactive metabolites which then bound to RSV microsomal protein. Furthermore, two of the human lung carcinoma cell lines containing high PES activity are susceptible to mitoxantrone induced cytotoxicity. The degree of cytotoxicity induced by mitoxantrone correlates with the PES activity in these two cell lines.

The mechanism(s) of PES-mediated cooxidation and toxicity of antitumor agents and other xenobiotics in human lung carcinoma-derived cells are currently under investigation. The utilization of homogenous human lung carcinoma cells systems for systematic screening of anticancer drugs directed at the PES system may provide hope for achieving target cell mediated antineoplastic effectiveness in lung cancer therapy.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

The importance of bioactivation of xenobiotics to toxic intermediates is well recognized. These bioactivations have been shown to be critical for the expression of the cytotoxic and/or carcinogenic potential of many compounds. Work from several laboratories indicates that a variety of compounds undergo oxidative metabolism during prostaglandin biosynthesis in vitro (Marnett and Elnig, Rev. Biochem. Toxicol. 5: 135, 1983). The term cooxidation has been applied to the description of PES catalyzed metabolism of xenobiotics. Among the compounds oxidized are chemical carcinogens that require oxidative activation to express their carcinogenic potential such as polycyclic aromatic hydrocarbons, aromatic amines, aflatoxin and nitrofurans. Because PES is widely distributed in mammalian tissues, further investigation is required to determine whether the PES system can serve as an important oxidative pathway in the metabolism of xenobiotics especially in tissues that have a low content of cytochrome P-450. Due to the lack of availability of homogeneous cell populations rich in the PES system, the vast majority of studies investigating the role of cooxidation as a pathway of bioactivation have been conducted in subcellular tissue preparations. In the past year, we have characterized several human lung carcinoma cell lines which differ extensively in their PES activity (see Annual Reports Z01 07173-01 LETM and Z01 CM 07174-01 LETM). The selected human lung carcinoma cell lines for this study have varying PES activities listed in ascending order: NCI-H69, NCI-H128, NCI-H322, NCI-H358 and NCI-H460 (see Annual Reports Z01 CM 07173-01 LETM and Z01 CM 07174-01 LETM). Both NCI-H69 and NCI-H128 are non-small cell carcinoma lines, NCI-H322 and NCI-H358 are well differentiated adenocarcinomas, and NCI-H460 is derived from a large cell carcinoma. These human lung carcinoma cell lines were employed as models to explore the ability of the different human lung cancers to activate xenobiotics, especially antitumor agents, directed against the PES system in the hope of achieving a more selective therapeutic effectiveness.

Methods Employed:

Chemical. Anticancer agents employed in this study included NMF (NSC 3051), cytoxan (NSC 26271), PCNU (NSC 95466), AZQ (NSC 182986), Caracemide (NSC 253272) and mitoxantrone (NSC 301739). Other compound used were ipomeanol, o-bromophenol and 2-bromohydroquinone.

Preparation of ram seminal vesicle (RSV) microsomes. Frozen ram seminal vesicular glands were thawed slowly at 4°C overnight. The tissue was minced and homogenized with a Potter-Elvehjem type homogenizer in 4 volumes (w/v) of 0.1 M phosphate buffer containing 1 mM EDTA, pH 7.8. The homogenates were centrifuged at 15,000 g for 20 min and the supernatant was centrifuged at 100,000 g for 1 hr to harvest the microsomes.

Enzymatic incubation with ram seminal vesicle microsomes. Various ^{14}C-labeled substrates, NMF, cytoxan, PCNU, AZQ, ceracemide, mitoxatrone, (at a concentration of 15 μM, 1.5 x 10^5 dpm/nmole), ^{3}H-ipomeanol, ^{14}C-o-bromophenol and ^{14}C-2-bromohydroquinone (at a concentration of 100 μM, 2200 dpm/nmole), were incubated with 0.08 mM arachidonic acid, 2 mg of RSV microsomes, 0.1 M phosphate buffer, 1 mM EDTA, pH 7.4 in a final volume of 1.5 ml at 37°C for 10 min. Incubations were terminated by transferring the mixture into 1 ml of 10% TCA.

The covalent binding of the radio-labeled reactive metabolites of the above substrates to RSV microsomal protein was determined.

Cytotoxicity study in human lung carcinoma cell lines. Human non-small cell carcinoma cell lines, NCI-H322, NCI-H358 and NCI-H460 were grown as monolayers in 35 cm^2 disk. The small cell carcinoma cell lines, NCI-H69 and NCI-128 were grown as suspension cultures. The culture medium, RPMI 1640 was removed from cell suspensions and monolayers and the cells washed 2 times with 5 ml of the same medium. Mitoxantrone, in concentrations ranging from 0.25-1.0 mM was added to the wells and the final volume was adjusted to 2 ml with RPMI 1640. The cell mixtures was incubated at 37°C in an atmosphere of 95% air/5% CO_2 for 6 hr. At each hour, 0.1 or 0.2 ml of the incubation medium was removed for LDH leakage determination.

Major Findings:

It has been demonstrated that certain types of reactions can be catalyzed by PES. These include N- and O-demethylation, epoxidation, S- and N-oxidation, C-hydroxy-lation and dehydrogenation (Marnet and Eling, Rev. Biochem. Toxicol. 5: 135, 1983). In the search for appropriate substrates for the PES system, a variety of compounds, different in chemical structure, were incubated with RSV microsomes (which contain high levels of PES). Of all the compounds tested (see above "Chemicals" section), only three showed significant covalent binding. These were mitoxantrone, o-bromophenol and 2-bromohydroquinone (specific activities: 8.8 ± 1.3, 21.8 ± 3.7, and 46.4 ± 8.4 nmole/mg protein/10 min, respectively). These results suggest that PES may play a role in the activation of mitoxantrone (an anticancer agent), o-bromophenol and 2-bromohydroquinone (both nephrotoxins) to reactive species.

Toxicity Study. When NCI-H322 and NCI-H358 cells were exposed to mitoxantrone in vitro, a time and dose dependent cytotoxicity measuring leakage of the cytosolic enzyme LDH was evident. Furthermore, the NCI-H358 cell line which possesses higher PES enzyme activity (2-fold) than the NCI-H322 cell line is more susceptible to the cytotoxic effect of mitoxantrone. This effect was both dose and time dependent. For example, 6 hr from the beginning of exposure to 1.0 mM of drug, >66% of LDH leakage in NCI-H358 cells and >38% of LDH leakage in NCI-H322 cells were observed. Furthermore, the dosage required to produce a similar LDH leakage over a period of 6 hr was twice as high for NCI-H322 cells as for NCI-H358 cells. Although the mechanism of cytotoxicity produced by mitoxantrone in these cell lines is not clear, these results suggest a correlation between the PES activity and the cytotoxicity of mitoxantrone. Appropriate inhibitors, such as aspirin, indomethacin, propylthiouracil, and stimulators such as calcium ionophore A23187 will also be used to further examine the possible role of PES in bioactivating mitoxantrone to its cytotoxic species.

Proposed Course:

Studies are planned to continue the characterization of the role of PES in xenobiotic metabolism, especially in the human lung carcinoma cell lines. The metabolism and the cytotoxic effects of mitoxantrone, o-bromophenol and 2-bromohydroquinone in NCI-H322 and NCI-H358 cells as well as in other human lung carcinoma cell lines including NCI-H460 (very high in PES activity), NCI-H69

and NCI-H128 (both with negligible PES activity) will be examined. The effects of PES inhibitors on the metabolism and cytotoxicity of mitoxantrone, o-bromophenol, and 2-bromohydroquinone, as well as the mechanisms of toxicity induced by these agents in these human lung carcinoma cell lines will be investigated.

Significance to Biomedical Research and the Program of the Institute:

We have demonstrated that the PES system isolated from ram seminal vesicles is capable of activating the anticancer agent, mitoxantrone and the nephrotoxins, o-bromophenol and 2-bromohydroquinone to covalently bound material during prostaglandin biosynthesis. Furthermore, two of the human lung carcinoma cell lines containing high PES activity are susceptible to mitoxantrone induced cytotoxicity. A systematic screening of anticancer drugs as well as model compounds directed at the PES system may provide the hope of achieving more selective therapeutic effectiveness in lung cancer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07168-01 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Biochemical Toxicology of the Nitrosoureas and the Thiol Modulating Agent, BSO

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	R. A. Kramer	Staff Fellow	LETM, NCI
Others:	M. R. Boyd	Associate Director	DTP, NCI
	D. J. Reed	IPA	LETM, NCI
	H. M. Schuller	Vet. Med. Officer	LETM, NCI
	J. H. Dees	Cancer Expert	LETM, NCI
	M. G. McMenamin	Biologist	LETM, NCI
	K. E. Greene	Bio. Lab. Tech.	LETM, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pharmacology and Toxicology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	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 objective of this project is to understand the biochemical mechanisms for the target organ toxicity of anticancer drugs. These studies have focused on two main areas of research: 1) the first project deals with the mechanism for the renal toxicity of MeCCNU and related nitrosoureas (i.e., CCNU, BCNU, chlorozotocin); and 2) the second study involves investigations on the use of buthionine sulfoximine as a thiol modulating agent in cancer chemotherapy.

Studies completed this year have demonstrated that: a) carbamylation-mediated reactions may not be necessary for nephrotoxicity to develop following the administration of nitrosourea anticancer agents; b) the alkylation of kidney DNA may be an important cellular target for nitrosourea nephrotoxicity; c) glutathione plays an important protective role against MeCCNU-induced renal toxicity; d) hepatic drug metabolizing enzymes can activate MeCCNU and CCNU, but not PCNU, to more polar and more reactive alkylating intermediates which can bind macromolecules in vitro; e) a liver-derived metabolite of MeCCNU may be responsible for the renal toxicity of the parent nitrosourea; f) in vivo administration of buthionine sulfoximine markedly reduced normal tissue, and L-PAM resistant L1210 tumor glutathione levels following a single dose of BSO, or after a multiple dosing regimen; g) of the normal tissues, the kidney was the most sensitive, and the bone marrow the most resistant, to thiol depletion by BSO; h) BSO increased the renal and hepatic toxicity of MeCCNU in F344 rats and increased the lethality of L-PAM in BDF mice.

Objectives:

1. Utilize the knowledge gained from an understanding of the biochemical mechanisms for the target organ toxicity of anticancer agents to aid in the rational design of better, less toxic, chemotherapeutic agents.

2. Develop improved methods for predicting, monitoring or treating such toxic reactions in patients.

3. Develop strategies for targeting tumor tissue, and minimizing host toxicity, to anticancer agents by the selective manipulation of tissue GSH levels.

I. Nephrotoxicity of the Nitrosoureas. Nephrotoxicity severely limits the therapeutic usefulness of a number of anticancer drugs (e.g., nitrosoureas, cis-platinum, mitomycin C). Renal toxicity was noted in preclinical studies for virtually all the nitrosoureas in clinical use. However, only recently have certain of these agents (i.e., MeCCNU) been shown to result in a delayed, irreversible and often fatal renal toxicity. The goals of this project have been to: a) develop an in vivo model for the study of nitrosourea nephrotoxicity (R.A. Kramer and M.R. Boyd, J. Pharmacol. Exp. Ther. 22: 409, 1983; Z01 CM 07139-02 LETM); and to b) elucidate the biochemical mechanisms for the renal toxicity of this class of antiatumor drugs (Z01 CM 07138-02 LETM).

Major Findings:

Nephrotoxicity of the Chloroethylnitrosoureas is Independent of Carbamylating activity. Most chloroethylnitrosoureas can degrade in solution to form carbonium ion alkylating agents in addition to isocyanates that are capable of carbamylation reactions. Whereas, antitumor activity is believed to be due to the aklylation of nucleophilic sites in DNA, carbamylation reactions have been implicated in mediating the host toxicity of the parent nitrosourea. Chlorozotocin is a glucopyranosyl conjugated chloroethylnitrosourea that represents the first clinically studied analog designed for low carbamylating activity. At equimolar doses, chlorozotocin was found to be a much more potent nephrotoxicant than MeCCNU. At low doses, both drugs produced a similar chronic and progressive nephropathy which was delayed in onset and resulted in karyomegaly to the collecting ducts of the renal medulla.

DNA Alkylation and Nephrotoxicity of the Chloroethylnitrosoureas. Renal DNA was found to be the major target for reactive alkylating intermediates derived from MeCCNU. Approximately 30% of the total drug in kidney was covalently bound to DNA; this compares to only 7% being protein bound. Kidney DNA bound 2.5- and 30-fold more MeCCNU than in the liver or the lung, respectively. The administration of MeCCNU resulted in an increase in kidney DNA (μg DNA/g kidney) which was proportional to the initial levels of drug bound covalently to DNA. Moreover, treatment with MeCCNU, or chlorozotocin, produced extensive karyomegaly to the collecting ducts of the renal medulla. These studies suggest that it may not be possible to dissociate the antitumor activity of the nitrosoureas (i.e., DNA alkylation), from their potential for nephrotoxicity.

Protective Role of GSH Against MeCCNU-induced Renal Toxicity. Treatment of F344 rats with MeCCNU resulted in a time- and dose-related decrease of GSH

preferentially in liver. Administration of MeCCNU to rats pretreated with buthionine sulfoximine (a selective inhibitor of glutathione biosynthesis) resulted in: a depletion of hepatic and renal GSH which was additive to the effects of either of these treatments alone; an increase in the concentration of reactive intermediates of MeCCNU which became irreversibly bound to renal and hepatic macromolecules; increased the nephrotoxicity of MeCCNU, and caused hepatotoxicity that was not ordinarily seen when MeCCNU was administered alone. MeCCNU was not found to effect glutathione reductase activity, or the ratio of GSH/GSSH, in any tissue tested. This differs considerably from the pneumotoxic nitrosourea, BCNU, which selectively effects these parameters within its target organ, the lung.

Metabolic Activation of MeCCNU. MeCCNU was shown to be metabolized by the hepatic monooxygenase system to reactive alkylating intermediates which bound irreversibly to macromolecules in vitro. This reaction met the criteria for a cytochrome P-450 dependent reaction, however, kidney microsomes were not found to catalyze this alkylation reaction. CCNU, but not PCNU, also was found to be activated by the liver monooxygenase system. Phenobarbital pretreatment resulted in a 20-fold increase in the alkylation of microsomal protein by MeCCNU; the alkylating metabolite accounting for up to 25% of the total metabolite profile of MeCCNU. In contrast, the carbamylating activity of MeCCNU was not significantly affected by the monooxygenase system. Liver microsomes were found to metabolize MeCCNU to a metabolite that was toxic to renal slices in vitro (i.e., renal slice organic ion transport; see Annual Report Z01 CM 07139-01 LETM). Addition of GSH to this in vitro reaction protected against the metabolite-dependent decrease in renal slice organic ion uptake and completely inhibited covalent binding to liver microsomes. GSH and other soluble nucleophiles (N-acetylcysteine, cysteine) also were found to inhibit covalent binding, and three N-acetylcysteine-MeCCNU metabolite adducts have been separated by HPLC.

Relationship Between Hepatic Metabolism and the Renal Toxicity of MeCCNU. In vivo studies demonstrated that the same enzyme systems involved with the metabolic activation of MeCCNU in vitro, may be involved in the metabolic activation and renal toxicity of MeCCNU in vivo. Inducers and inhibitors of these enzymes markedly altered both the in vivo covalent binding and the renal toxicity of MeCCNU. Pretreatment with phenobarbital, an inducer of the monooxygenase system in liver but not in kidney, increased both the alkylation and toxicity of MeCCNU in the kidney. Moreover, an in vivo/in vitro toxicity assay demonstrated the presence of a metabolite in the bile of a MeCCNU treated rat which was toxic to cells in vitro.

Chloroethyl-GSH Adducts. The exact chemical nature of the putative liver-derived nephrotoxic metabolite is not known. However, it is possible that hepatic metabolism of MeCCNU could lead to the formation of a 2-chloroethyl-GSH adduct (see Annual Report Z01 CM 07159-01 LETM). Studies initiated this year have shown that 2-chloroethyl-GSH is a potent nephrotoxicant, presumably via the formation of a reactive episulfonium ion. Moreover, we have found that this reactive GSH adduct may act by a different mechanism than other known nephrotoxic GSH-adducts (e.g., dichlorovinyl-GSH; bromohydroquinone-GSH). These compounds are dependent upon the enzyme γ-glutamyltranspeptidase (γ-GT)

for transport into proximal tubule cells where they ultimately exert their toxicity. In contrast, 2-chloroethyl-GSH was nephrotoxic by a γ GT independent mechanism, providing evidence that the kidney can process GSH-adducts to form their corresponding mercapturic acids by more than one mechanism.

II. Thiol Modulation. GSH plays a crucial role in the cellular protection against a variety of toxicologic insults, including reactive oxygen intermediates, free radicals, lipid peroxides and reactive electrophiles. The overall goal of this project is to develop strategies that would maximize thiol depletion in tumor tissue, and minimize the possible host toxicity resulting from combination therapies involving thiol depletion. These studies have initially focused on buthionine sulfoximine (BSO), a selective inhibitor of the rate-limiting enzyme in GSH biosynthesis (i.e., γ-glutamyl cysteine synthetase).

Major Findings:

1. Adminisration of BSO to F344 rats or BDF mice dramatically reduced tissue GSH concentrations.

2. Large differences were observed between tisues with respect to the rate of GSH depletion and recovery, and the maximum level of thiol depletion obtained following a single dose of BSO. In this regard, the kidney was the most sensitive organ for thiol depletion by BSO (20% of control), and the bone marrow was the most resistant (85% of control).

3. In vivo administration of a single dose of BSO led to a 75% decrease in GSH levels in L-PAM resistant L1210 in BDF mice. Multiple dosing regimens of BSO led to an even further decrease in tissue and tumor GSH levels. Tumor GSH levels being the most depleted following multiple doses of BSO (10-fold decrease).

4. BSO pretreatment increased the renal and hepatic toxicity of MeCCNU in F344 rats and increased the lethality of L-PAM in BDF mice.

Publications:

1. Kramer, R.A., Schuller, H.M., Smith, A.C. and Boyd, M.R.: Effects of buthionine sulfoximine on the nephrotoxicity of 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (MeCCNU). J. Pharmacol. Exp. Ther., in press.

2. Kramer, R.A., McMenamin, M.G. and Boyd, M.R.: Differential distribution and covalent binding of two labeled forms of 1-(2-chloroethyl)-3-(trans-4-methylcyclohexyl)-1-nitrosourea (Methyl CCNU) in the Fischer 344 rat. Cancer Chemother. Pharmacol. 14: 150-155, 1985.

3. Kramer, R.A., McMenamin, M.G. and Boyd, M.R.: Mechanism of chloroethyl-nitrosourea nephrotoxicity: Studies with MeCCNU. In Bach, P.H. and Lock, E.A. (Eds.): Renal Heterogeneity and Target Cell Toxicity. John Wiley & Sons, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07169-01 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Tetraplatin Toxicity: Comparative Nephrotoxicity of Platinum Anticancer Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	J.H. Smith	PRAT Staff Fellow	NIGMS
Others:	M.A. Smith	Staff Fellow	LETM, NCI
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	J. Uozumi	Visiting Fellow	LPET, NCI
	S. J. Lovett	Bio. Lab. Aid	LETM, NCI
	T. MacLain	Bio. Lab. Tech.	LPET, NCI
	M. R. Boyd	Associate Director	DTP, NCI

COOPERATING UNITS *(if any)*

U.S. Environmental Protection Agency (M. P. Copley)

LAB/BRANCH

Laboratory of Experimental Therapeutics & Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	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.)*

Tetraplatin (NSC 363812) is a new anticancer platinum drug analog targeted for clinical development because of its effectiveness against cisplatin-resistant tumor cell lines and its improved formulation. The toxicity of tetraplatin was assessed in Fischer 344 rats and compared to equimolar doses of cisplatin and CHIP (NSC 256927). This emphasis of this investigation was the comparison of the relative nephrotoxicity of these platinum analogs and the relative sensitivity of various tests used to monitor renal function. At equimolar doses tetraplatin was less nephrotoxic than cisplatin. Furthermore, the characteristics of tetraplatin nephrotoxicity differed markedly from cisplatin. Most notably, a severe diuresis occurred at the dose of tetraplatin where indications of nephrotoxicity were first detected. In contrast to cisplatin, effects on proximal tubular cell function assessed in renal cortical slices were less severe. Histologically, the tetraplatin lesion extended throughout the renal cortex and no frank tubular necrosis was observed. Cisplatin lesions were characteristically localized in the corticomedullary junction and were more severe. Urinary excretion of proteins, glucose and enzymes was the most sensitive indicator for the onset of nephrotoxicity. BUN and serum creatinine were not elevated until the renal damage was more severe at higher doses. There appear to be other differences between the toxicity of tetraplatin and cisplatin relating to hematologic disorders and gastrointestinal toxicity.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives:

Nephrotoxicity is the major dose-limiting factor for the therapeutic usefulness of cisplatin. A multitude of cisplatin analogs have been evaluated for effectiveness against cisplatin-resistant tumor cell lines and for improved formulation (purity, solubility, and stability). Tetrachloro(d,1-trans)1,2-diaminocyclohexane platinum (IV) (NSC 363812, Tetraplatin) was recommended for development to clinical trial by the Platinum Analog Working Group at the March 20, 1984 meeting of the Decision Network Committee. It was recommended that the nephrotoxicity of tetraplatin be evaluated before passing this drug to the Decision Point 2B Status.

The purpose of this investigation was to evaluate the nephrotoxic potential of tetraplatin. As a basis for the comparison, equimolar doses of cisplatin and CHIP (NSC 256927) were compared to tetraplatin in the Fisher 344 rat. A second goal of this investigation was to evaluate the sensitivity of noninvasive parameters of renal function and toxicity in order to better monitor for the onset of nephrotoxicity in the clinic.

Methods:

Adult male Fischer 344 rats (10 wk) received a single, i.v. bolus injection of saline, cisplatin, CHIP or tetraplatin in a volume of 4 ml/kg at equimolar doses of drugs. The doses were 6.7, 13.4, 26.7 or 53.3 μmol/kg and were equivalent to 2, 4, 8 and 16 mg/kg of cisplatin, respectively. Injections were made between 0800 to 0930 hr. Rats were killed on days 1, 3, 5, 8 and 15 for assessment of toxicity. There were 4 replicate rats per dose and time point. The emphasis of this investigation was the comparison of the relative nephrotoxicity of platinum analogs and the relative sensitivity of various tests used to monitor renal function. Therefore, rats to be killed on day 15 were housed in stainless steel metabolism cages and urine was collected daily on ice. Urine was analyzed for total daily urine volume, osmolality, pH, protein, Pt concentration and urinary enzymes. Lactate dehydrogenase (LDH) excretion was measured as an example of a cytosolic enzyme and N-acetyl-β-glucosamindase (NAG) excretion was measured as a representative lysosomal enzyme. Enzyme excretions monitored as representatives of brush border damage (and thus proximal tubular damage) included alanine aminopeptidase (AAP), alkaline phosphatase (AP), γ-glutamyltranspeptidase (γ-GT) and maltase. In addition, the following parameters were assessed in rats killed on day 1, 3, 5, 8 and 15.

1 - The change of body weight from the day of injection.

2 - Blood was collected for the vena cava for:

a - complete blood counts on whole blood; and for

b - serum analysis of urea (BUN), creatinine, electrolytes, (Ca^{2+}, Cl^{-}, K^{+}, Mg^{2+}, Na^{+}) and total Pt concentration.

3 - An aliquot of urine was likewise analyzed for electrolytes.

4 - Kidneys were analyzed for Pt and total glutathione concentration.

Renal cortical slices were prepared for in vitro assessment of proximal tubular function which included organic ion accumulation and gluconeogenesis. Additionally, samples of kidney, liver and duodenum were prepared for histopathological examination.

Major Findings:

At equimolar doses, tetraplatin was less nephrotoxic than cisplatin. At a dose of 6.7 μmol/kg there was no evidence of tetraplatin nephrotoxicity, in contrast to rats receiving this dose of cisplatin.

Furthermore, the characteristics of tetraplatin nephrotoxicity differed markedly from those of cisplatin. Most notably, 13.3 μmol/kg of tetraplatin produced a striking 10-20 fold elevation of daily urine volume; rats were voiding up to 100 ml of urine per 24 hr. This was accompanied by a corresponding increased excretion of protein, glucose, urinary enzymes and K^+. Proximal tubular cell function assessed in renal cortical slices was not markedly affected in rats treated with tetraplatin. In contrast, 13.3 μmol/kg of cisplatin decreased organic ion accumulation and gluconeogenesis and resulted in small elevations of glutathione concentrations in renal cortical slices. Urinary excretion of brush border-derived enzymes after both cisplatin and tetraplatin indicated damage to the proximal tubular portion of the nephron. Histopathological examination of kidneys confirmed differences between cisplatin and tetraplatin nephrotoxicity. Renal lesions after cisplatin primarily were localized to the corticomedullary junction and were much more severe than those of tetraplatin, where the lesion occurred more diffusely throughout the renal cortex. The extent of cellular and tubular degeneration and necrosis was much more severe after cisplatin. In contrast to cisplatin, no giant cells nor frank tubular necrosis was observed with tetraplatin, even at the highest dose of 53.3 μmol/kg. No indication of nephrotoxicity was detected in rats treated with CHIP, another Pt(IV) analog, except at this highest dose of 53.3 μmol/kg.

Urinary analysis for excretion of protein, glucose and enzymes was the most sensitive indicator for the onset of nephrotoxicity for all platinum drugs evaluated. BUN and creatinine were elevated slightly (3-fold) 5 d after 26.7 μmol/kg of tetraplatin and by 10-fold after 53.3 μmol/kg of tetraplatin. Marked elevations of urinary protein and enzyme excretion were detected after 13.3 μmol/kg of tetraplatin. This sequence was observed in cisplatin and CHIP treated rats as well.

There appear to be other differences between the toxicity of tetraplatin and cisplatin. Decreases of white blood cell count and platelets were detected with the lowest doses of tetraplatin, in contrast to cisplatin. Furthermore, characteristics of gastrointestinal toxicity differed with tetraplatin. Little or no feces was apparent in metabolism cages, food consumption appeared to be decreased, and symptoms of a hemorrhagic stomach were observed on kill dates in rats treated with 26.7 μmol/kg tetraplatin. At the highest dose, rats exhibited a severe watery diarrhea and stomachs were noted to be enlarged and fluid-filled.

Significance to Biomedical Research and the Program of the Institute:

The nephrotoxic potential of a new and promising platinum analog anticancer agents was evaluated in a novel protocol. The nephrotoxicity was compared at depth with cisplatin, the platinum drug most widely used in the clinic to date. Data obtained from this investigation will be useful in designing further toxicologic investigations of tetraplatin and other platinum drug analogs, and in monitoring and reducing the incidence of nephrotoxicity if this drug is evaluated clinically. These data strongly indicate that monitoring of BUN or creatinine is inappropriate for assessing the onset of nephrotoxicity after this class of anticancer drugs.

Proposed Course:

Tetraplatin has been pased to Decision Point 2B by the Decision Network committee and will now enter full-scale preclinical toxicity evaluation. These platinum drug analogs are being evaluated currently in several in vitro models for assessing nephrotoxicity, including renal cortical slices (see Annual Report Z01 CM 07160-02 LETM) and isolated renal epithelial cells. Upon the availability of human renal tissue, these compounds will be evaluated in similar in vitro systems in an attempt to predict the nephrotoxic potential of tetraplatin to humans.

Publications:

1. Litterst, C., Smith, J.H., Smith, M.A., Uozumi, J. and Copley, M.: Sensitivity of urinary enzymes as indicators of renal toxicity of the anticancer durg cisplatin. In Bianchi, C. and Blaufox, D.M. (Eds.): Proceedings from Newer Diagnostic Methods in Nephrology and Urology. Basel, Switzerland, Karger Publ. Co., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07171-01 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Thiol-Promoted Lipid Peroxidation in Kidney Microsomes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: E. G. Mimnaugh Chemist LETM, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	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.)*

Although reduced glutathione (GSH) is known to have an inhibitory (antioxidant) effect on lipid peroxidation in microsomes from liver in vitro and is thought to play an important role in controlling lipid peroxidation in vivo through the action of glutathione peroxidase, it was observed that, in contrast, GSH dramatically enhanced NADPH-dependent lipid peroxidation in rodent kidney microsomal fractions. This unexpected result was studied further with the aim of understanding the mechanism by which GSH and other thiols promoted lipid peroxidation. This process could have toxicological importance in kidney in that it may amplify NADPH-dependent lipid peroxidation.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

The enzymatic diversion of reducing equivalents from thiols to promote the peroxidation of membrane lipids appears to be a previously unrecognized mechanism of lipid peroxidation. The objectives of this study are to characterize the biochemistry of this process through the use of inhibitors and altered incubation conditions, to investigate the apparent organ-specificity of thiol-promoted peroxidation, to attempt to identify the enzyme, and to determine if the process has toxicological relevancy.

Methods:

Microsomal fractions are isolated from kidney homogenates by differential centrifugation. Lipid peroxidation is assayed by the thiobarbituric acid method. The conversion of GSH to GSSG or other oxidative products will be determined by HPLC. The participation of oxyradicals in thiol-promoted lipid peroxidation will be assessed by the use of specific scavengers of reactive oxygen species or by direct assay.

Major Findings:

Lipid peroxidation supported by GSH was concentration (1 to 10 mM) and time-dependent, correlated with GSH oxidation, and was synergistic with either NADPH or NADH. The process appeared to be enzymatic in that the effect could be completely blocked by heat-inactivating the kidney microsomes, and to be metal ion-dependent (Cu^{2+}, Fe^{2+} and Fe^{3+}). Other thiols could substitute for GSH and these included: cysteine, N-acetylcysteine, cysteamine, dithiothreitol and dimercaprol, but S-methyl glutathione could not support peroxidation. Lipid peroxidation in the presence of GSH and NADPH could be diminished by scavengers of oxyradicals and by metal ion chelators as well as by KCN. It was noted that adriamycin-stimulated kidney NADPH-dependent microsomal lipid peroxidation could be further enhanced by GSH. These preliminary results suggest that in kidney, GSH-enhanced lipid peroxidation is an enzyme-mediated process which may utilize reducing equivalents derived from thiol oxidation.

Significance to Biomedical Research:

This process may have toxicological importance in that it can amplify NADPH- or NADH-dependent peroxidation of unsaturated membrane lipids.

Proposed Course:

Glutathione-promoted peroxidation appears to be kidney-specific and enzyme-mediated. The identity of this thiol-dependent enzyme is unknown. Future experiments will be designed to characterize the enzyme.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07172-01 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Role for Reactive Oxyradicals and Lipid Peroxidation in Anthracycline Cytotoxicity

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

P.I.: E. G. Mimnaugh Chemist LETM, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	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.)*

Recent reports support the hypothesis that a mechanism by which anthracyclines cause cardiotoxicity involves the formation of drug free radical and enhanced oxyradical production. It is possible that at least a portion of the antitumor activity of anthracyclines may also result from anthracycline-mediated oxyradical production leading to damage to intracellular membranes through the process of lipid peroxidation. Information relevant to this possibility is presently lacking, and, therefore, this project was designed to evaluate the oxyradical hypothesis of cytotoxicity with isolated microsomal and mitochondria subcellular fractions from several model tumors.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives:

Previous studies from this and other laboratories have shown that either microsomes or mitochondria from various tissues, in the presence of a suitable reducing agent, are capable of metabolically activating Adriamycin by a one electron reduction to an unstable semiquinone free radical. The Adriamycin semiquinone intermediate subsequently reacts with molecular oxygen to generate superoxide radical, hydrogen peroxide, and ultimately hydroxyl radical, which initiate and propagate the peroxidation of polyenoic membrane phosphoglycerides. Membrane lipid peroxidation is a destructive process in that it disrupts membrane structure and function, inactivates membrane-bound enzymes, generates reactive lipid-derived aldehyde products, and ultimately, leads to cell death. The objectives of this study are to determine whether the above described biochemial process contributes to the antitumor activity of anthracyclines, and to describe those factors which participate in the process.

Methods Employed:

Tumors are grown either in cell culture or in vivo in mice, and microsomal and mitochondrial subcellular fractions are isolated from tumor homogenates. These fractions contain the enzymes capable of metabolically activating anthracyclines (NADPH-cytochrome P-450 reductase and NADH-dehydrogenase, respectively) and the membrane unsaturated lipids, which are targets for oxyradical attack. Incubations are conducted in the presence of Adriamycin and lipid peroxidation is assessed by the thiobarbituric acid method. Enzymatic assays are conducted, and oxyradical scavengers are employed to delineate the mechanism of lipid peroxidation.

Major Findings:

Adriamycin stimulated NADPH-dependent lipid peroxidation 4 to 7-fold in microsomes isolated from EMT6 mammary carcinoma cells and from B-16 solid tumors. The maximal enhancement of lipid peroxidation in microsomes from B-16 melanoma was observed with adriamycin at 100 μM, with exogenously added ferrous iron at 10 μM and in incubations conducted under a 100% oxygen atmosphere. Microsomal NADPH-cytochrome P-450 reductase activities, which could contribute reducing equivalents for lipid peroxidation, were 56 ± 67 and 28 ± 6 nmoles of cytochrome c reduced/mg protein/min for EMT6 and B-16 tumors, respectively. Adriamycin failed to enhance NADPH-dependent lipid peroxidation in either EMT6 or B-16 melanoma mitochondria.

Significance to Biomedical Research and the Program of the Institute:

Elucidation of the mechanisms of tumor cell killing by anthracyclines will result in a better understanding of the biomedical toxicology of this class of antitumor drugs and provide a basis for the rational design of drugs which utilize reactive oxyradicals in cytotoxic chemotherapy.

Proposed Course:

Further work is planned to investigate the participation of superoxide, hydrogen peroxide and hydroxyl radical in adriamycin-stimulated tumor microsomal lipid

peroxidation, and to extend the study to include other anthracycline-sensitive tumors.

Publications:

1. Sinha, B.K., Trush, M.A., Kennedy, K.A. and Mimnaugh, E.G.: Enzymatic activation and binding of adriamycin to nuclear DNA. Cancer Res. 44: 2892-2986, 1984.

2. Mimnaugh, E.G., Trush, M.A., Bhatnagar, M. and Gram, T.E.: Enhancement of reactive oxygen-dependent mitochrondrial membrane lipid peroxidation by the anticancer drug, adriamycin. Biochem. Pharmacol. 34: 847-856, 1985.

3. Mimnaugh, E.G., Kennedy, K.A., Trush, M.A., and Sinha, B.K.: Adriamycin-enhanced membrane lipid peroxidation in isolated nuclei. Cancer Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07173-01 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Arachidonic Acid Metabolism in Human Lung Adenocarcinoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. S. Lau	Sr. Staff Fellow	LETM, NCI
Others:	J. B. McMahon	Cancer Expert	LETM, NCI
	H. M. Schuller	Vet. Med. Officer	LETM, NCI
	M. R. Boyd	Associate Director	DTP, NCI
	M. G. McMenamin	Chemist	LETM, NCI
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COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pharmacology and Toxicology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	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.)*

To date, there have been no reports linking arachidonic acid (AA) metabolism with the pathobiology of specific human tumor cell populations in pulmonary adenocarcinoma (PAC). The correlation of prostaglandin (PG) biosynthesis with the pathobiology of PAC was studied by exploring the metabolism of AA in human lung carcinoma cell lines. Two PAC derived cell lines (NCI-H322 and NCI-H358) and two small cell carcinoma derived cell lines (NCI-H69 and NCI-H128) were used. No metabolism of AA occurred in either unstimulated or calcium ionophore A23187-stimulated NCI-H69 and NCI-H128 cells. The major metabolite of AA isolated from both NCI-H322 and NCI-H358 cells was identified as prostaglandin E_2 (PGE_2). The prostaglandin endoperoxide synthase (PES) activity expressed as immunoreactive PGE_2, (pmole/min/mg protein) was found to be 10.3 ± 0.28 and 4.8 ± 0.48 in NCI-H358 and NCI-H322 cells, respectively. Production of PGE_2 in both NCI-H358 and NCI-H322 cells was linear up to 10 min with apparent Km of 8.3 and 5.5 μM and Vmax of 12.5 and 5.3 pmole/min/mg protein for NCI-H358 and NCI-H322 cells, respectively. Moreover, PGE_2 did not undergo further metabolism by either the NCI-H358 nor the NCI-H322 cells. Aspirin, inhibited PGE_2 production by 78% and 60% in NCI-H358 and NCI-H322 cells, respectively. In the presence of exogenous AA, A23187 stimulated PGE_2 production in NCI-H322 cells by 76% without affecting the production of PGE_2 in the NCI-H358 cells. In contrast, A23187 stimulated the endogenous production of PGE_2 to a greater extent in both NCI-H322 and NCI-H358 cells. These results suggest that certain types of tumors within the category of human PAC may preferentially synthesize and release PGE_2. This finding may be useful diagnostically since elevated PGE_2 synthesis may be a characteristic unique to a group or subclass of PAC in patients. Furthermore, the human PAC lines can be employed as models to explore the ability of the different human lung cancers to activate xenobiotics, especially antitumor agents directed at the PES system in hopes of achieving more selective therapeutic effectiveness.

GPO 914-918

Objectives:

The pathobiology of human lung cancer is often associated with the biosynthesis and release of various biologically active substances by malignant cells. A number of studies have indicated that prostaglandins (PGs) and other eicosanoids may play a role in some aspects of the pathobiology of malignant disease, such as tumor promotion, cellular proliferation and differentiation. This study is initiated to determine whether cancer cells produce high levels of PGs, and if specific cell types are responsible for such reactions. We have used various well established and morphologically well characterized human lung cancer cell lines (see Annual Report Z01 CM 07175-01 LETM). For these studies, four human lung carcinoma cell lines were chosen. Two are well differentiated PAC, NCI-H322 and NCI-H358. The other two are established small cell carcinoma lines (SCCL), NCI-H69 and NCI-H128. Ultrastructurally, NCI-H322 possesses rough and smooth endoplasmic reticulum and electron-dense secretion granules which are characteristic of bronchiolar Clara cells. The NCI-H358 cells possess endoplasmic reticulum and well-defined lamellar bodies, hallmark structures of alveolar type II cells. Both SCCL, NCI-H69 and NCI-H128, are essentially devoid of endoplasmic reticulum and express features of endocrine cells. In this report, we present details of our findings on the metabolism of AA in these human lung carcinoma cell lines.

Methods Employed:

Cell culture. The two PAC (NCI-H322 and NCI-H358) were grown as monolayers in 75 cm^2 plastic flasks. They were maintained in RPMI 1640 medium supplemented with glutamine (2 mM), FBS (10% v/v) and gentamycin (50 mg/ml) at 37°C in an atmosphere of 95% air/5% CO_2. The cells were subcultured weekly and the medium was replaced by fresh medium every 3 days. The cultures were used when the monolayer had reached near confluency (about 6-8 days). The two SCCL (NCI-H69 and NCI-H128) were grown in suspension and maintained as described above.

Incubation. Prior to the incubation the cells were washed three times with 20 ml of Hank's balanced salt solution (without Ca^{++} and Mg^{++}). Three ml of Hank's balanced salt solution containing calcium (1.5 mM) were then added to either suspend the cells or to cover the monolayers. Three µCi of 1-^{14}C-AA (60 mCi/mmol) were added to each flask and incubated for 35 min. In other studies in which the calcium ionophore A23187 (20 µM) was added, the cells were incubated at 37°C with or without ^{14}C-labeled AA for 10 min before the addition of the ionophore and 15 minutes subsequent to the addition of the ionophore. In studies in which aspirin was added to the cells, the cells were incubated with 0.1 mM aspirin for 10 min before the ^{14}C-labeled AA was added. In two other groups of studies, exogenous AA (1.4 to 33.2 µM) was added to monolayers of NCI-H322 and NCI-H358 cells and incubations were performed at 37°C for periods of time ranging from 0-30 min. The incubations were terminated by removal of the incubation medium from the suspensions or monolayers of cells.

Analysis of products: The incubation medium was removed from suspensions of SCCL (NCI-H69 and NCI-H128) and monolayers of PAC (NCI-H322 and NCI-H358) and analyzed by reversed phase high performance liquid chromatography (HPLC) for the presence of ^{14}C-labeled metabolites of AA. The medium removed from the

cells was evaluated for the presence of fatty acid cyclooxygenase and lipoxygenase products of AA. The ^{14}C-labeled products resolved by HPLC were directly compared with known standards of fatty acid cyclooxygenase and lipoxygenase products of AA. The identity of ^{14}C-labeled metabolites of AA isolated from the cell suspensions and monolayers was established via combined gas chromatography-mass spectrometry. Quantitation of the metabolite isolated from monolayers of NCI-H322 and NCI-H358 cells was achieved by radioimmunoassay (RIA).

Major Findings:

The major cyclooxygenase-peroxidase catalyzed ^{14}C-AA metabolite in these human carcinoma cell lines was identified as PGE_2. The lipoxygenase pathways of ^{14}C-AA in these human cell lines was also assessed by HPLC assay but no metabolite(s) could be detected under our assay conditions. NCI-H358 and NCI-H322 cells, both PAC, are capable of metabolizing ^{14}C-AA to PGE_2. In contrast, levels of PGE_2 in both the NCI-H69 and NCI-H128 cells, both SCCL, were nondetectable. Formation of PGE_2 in both the NCI-H322 and NCI-H358 cells was linear up to 10 min in the presence of exogenous AA (18 μM). The kinetic parameters for the metabolic transformation of AA were investigated in the NCI-H358 and NCI-H322 cell lines. Similar apparent Michaelis-Menten affinity constant (k_m) values for the formation of PGE_2 were obtained in NCI-H358 and NCI-H322 (8.3 and 5.5 μM, respectively). However, the rate of formation of PGE_2 was signi - ficantly higher in the NCI-H358 cells than in the NCI-H322 cells. Their apparent Vmax values were 12.5 and 5.3 pmole/min/mg protein, respectively. The PES activity expressed as immunoreactive PGE_2 in pmole/min/mg protein was found to be 10.3 ± 0.28 and 4.8 ± 0.48 in NCI-H358 and NCI-H322, respectively. Aspirin, a cyclooxygenase inhibitor, at 0.1 mM, inhibited PGE_2 production by 78% and 60% in NCI-H358 and NCI-H322 cells, respectively. In the presence of endogenous AA, the calcium ionophore A23187, at 20 μM, stimulated PGE_2 production in NCI-H322 cells by 76%, while it did not affect the production of PGE_2 in the NCI-H358 cells. In contrast, A23187 stimulated the endogenous production of PGE_2 to a greater extent in both NCI-H322 and NCI-H358 cells (3- and 8-fold, respectively). Furthermore, ^{3}H-PGE_2 was incubated with NCI-H358 and NCI-H322 cell lines to determine if they were capable of metabolizing PGE_2. No disappearance of ^{3}H-PGE_2 in the incubation medium in either of these two cell lines was observed.

Significance to Biomedical Research and the Program of the Institute:

We have demonstrated in the study that two PAC preferentially metabolize AA primarily to PGE_2. Moreover, these results show that within the category of human PAC, AA metabolism exhibits significant differences in individual cell types. This finding provides a tool for a more detailed examination of biochemical functions of these tumor cells in relation to the pathobiology of human lung cancer. The preferential biosynthesis of PGE_2 by certain types of lung cancer cell lines may be useful diagnostically, and in the determination of the effectiveness of antitumor therapy in human subjects. Furthermore, the human PAC lines can be employed as models to explore the ability of the different human lung cancers to activate xenobiotics, especially antitumor agents directed at the PES system, in hopes of achieving more selective therapeutic effectiveness.

Proposed Course:

It is well estabished that a variety of compounds undergo oxidative metabolism during prostaglandin biosynthesis in vitro. The ability of certain types of human lung carcinomas to synthesize prostaglandins may convey significant capability to such tumors for the metabolic activation of antitumor agents and other xenobiotics via P-450 monooxygenase-independent mechanism. Thus, studies of PES-mediated cooxidation of antitumor agents and other xenobiotics will be carried out in these human lung carcinoma-derived cells. Furthermore, the role of AA metabolism in tumor differentiation as well as metastases development in vivo will be examined in tumor-bearing nude mice after injection of homogenous carcinoma cells of early and late passages from individual cell lines.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07174-01 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Arachidonic Acid in Pulmonary Large Cell Carcinoma

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. S. Lau	Sr. Staff Fellow	LETM, NCI
Others:	H. M. Schuller	Vet. Med. Officer	LETM, NCI
	J. B. McMahon	Expert	LETM, NCI
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COOPERATING UNITS *(if any)*

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LAB/BRANCH

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SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.3	0.6	0.3

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Clinically, large cell carcinoma (LCC) is by far the least treatable lung cancer. Characterization of this subclass of carcinoma by an exclusive cell type is not possible even under electron microscopic examination. Thus, investigation of the biochemical and morphological features of human LCC is warranted. The pathobiology of human lung cancer is often associated with the biosynthesis and release of various biologically active substances by malignant cells. A number of studies have indicated that prostaglandins (PGs) and other eicosanoids may play a role in the pathobiology of malignant disease, i.e., tumor promotion, cellular proliferation and differentiation. One metabolite of arachidonic acid (AA), prostaglandin E_2 (PGE_2), has been implicated as a mediator of hypercalcemia associated with certain lung cancers (Seyberth at al., N. Engl. J. Med. 239: 1228, 1975). The leukotrienes participate in the initiation of immune and inflammatory responses (Lewis and Austen, J. Clin. Invest. 73:889, 1984) and thus could play a role in host defense mechanisms in human lung cancer. Studies of the metabolism of AA were undertaken to determine the pathways of AA metabolism and the relevance of this metabolism in human LCC. Our preliminary studies demonstrated that a homogenous human LCC line, NCI-H460, unlike any other known human lung carcinoma cell line maintained in this laboratory possesses significantly higher PGE_2 synthesis (6 fold) in the early passage (15) as compared to that in the late passage (93). Furthermore, the morphology of this cell line (see Annual Report Z01 CM 07175-01 LETM) has changed. Originally diagnosed as LCC (containing large amounts of endoplasmic reticulum), it was reclassified as a small cell carcinoma (exhibiting endocrine cell features) in passage 15 and it is now (passage 93) described as an oncocytoma (containing large numbers of mitochondria). The mechanism for such an alteration is unknown. To examine the role of AA metabolism in tumor development in vivo, cells from early and late passages of NCI-H460 will be injected into nude mice. The profile of AA metabolism in the primary tumor as well as in the metastases formation will be investigated.

Objectives:

The pathophysiology of human lung cancer has been attributed in part to the biosynthesis and release of different mediators by malignant cells. Because the products of AA possess potent biological activities and are preferentially synthesized by human non-small cell carcinoma of the lung, arachidonate metabolism may be relevant to human lung cancer-associated pathobiology. Seyberth and coworkers (N. Engl. J. Med. 239:1228, 1975) have demonstrated that production of PGE_2 is significantly elevated in certain populations of hyercalcemic cancer patients with primary pulmonary malignancies classified as squamous cell carcinomas. Bennett et. al. (Br. J. Cancer 46:888, 1982) reported that levels of prostaglandin-like material, bioassayable as PGE_2 activity, is significantly higher in human lung carcinoma tissue than in adjacent normal lung tissue after incubation in vitro. While these studies indicate that eicosanoid production is elevated in human lung carcinoma and fibrosarcoma tissues, and in squamous cell carcinoma of the lung, attempts to correlate a role of PGs and other eicosanoids in the pathobiology of human lung cancer have proven unrewarding. A major impediment in determining the role of PGs in the pathobiology of human lung cancer cell in the studies cited is the lack of availability of homogenous cell populations defined by cell type derived from human lung carcinomas. As a result, there is a paucity of information related to the metabolism of AA and other fatty acid precursors of eicosanoids in human lung carcinomas. To determine whether cancer cells produce high levels of PGs and whether specific cell types are responsible for such production, we have used various well established and morphologically well characterized human lung cancer cell lines (see Annual Report Z01 CM 07175-01 LETM). Some of the results of the metabolism of AA in various human lung cancer lines has been summarized in a separate report (see Annual Report Z01 CM 07173-01 LETM). Among these cell lines, NCI-H460 demonstrates a unique characteristic. Histopathologically, its tumor of origin was diagnosed as large cell carcinoma while the cell line derived from it demonstrated features of endocrine cells at early passage which subsequently changed to oncocytoma at late passage. The objectives of the present study are: 1) to compare the metabolism of AA in early and late passages in culture and 2) to investigate the role of AA metabolism in tumor development in nude mice after injection of homogenous NCI-H460 LCC.

Methods Employed:

Cell culture and incubations: NCI-H460 cells from passage 15 and passage 93 were grown as monolayers in 75 cm^2 plastic flasks. They were maintained in RPMI 1640 medium supplemented with glutamine, FBS and gentamycin, at 37°C in an atmosphere of 95% air/5% CO^2. Prior to the incubation with AA, cells were washed three times with 20 ml of Hank's balanced salt solution (without Ca^{++} and Mg^{++}). Three ml of Hank's balanced salt solution (HBSS) containing calcium (1.5 mM) were then added to cover the monolayers. Cells were incubated at 37°C for 10 minutes in the presence or absence of aspirin (0.1 mM); ^{14}C-AA (1 μCi, 40 μM) was then added and the cells were incubated at 37°C for 10 minutes. In studies in which the calcium ionophore A23187 (20 μM) was added, the cells were incubated at 37°C with or without exogenous AA for 10 minutes before the addition of ionophore and for 15 minutes subsequent to the addition of the ionophore. The incubations were terminated by removal of the HBSS from the monolayer cells.

Analysis:

The HBSS removed from the monolayers of the two passages of NCI-H460 cells was analyzed by HPLC for the presence of cyclooxygenase products. Quantation of PGE_2 was achieved by radioimmunoassay (RIA).

Major Findings:

The predominant cyclooxygenase product of AA was found to be PGE_2 in both early (15) and late (93) passages of NCI-H460 cell lines. The profile of AA metabolism measured as PGE_2 formation in the early (passage 15) and late (passage 93) passages of cell line NCI-H460 is substantially different in both rate and response to stimulator, such as A23187. These results are summarized in the following table.

	PGE_2 (p mole/min/mg protein)			
	Passage 15		Passage 93	
Additions	+ AA (40 μM)	-AA	+AA (40 μM)	-AA
none	37.5 ± 0.5(100%)	7.6 ± 0.2(100%)	6.3 ± 0.9(100%)	0.43 ± 0.03(100%)
+Asp(.1mM)	15.2 ± 1.7(40%)	N.D.	1.7 ± 0.1(22%)	N.D.
+A23187 (20 μM)	50.3 ± 2.4(134%)	40.1 ± 2.0(528%)	7.2 ± 0.4(115%)	0.86 ± 0.1(200%)

PGE_2 production is significantly higher in passage 15 than in passage 93 in the presence and absence of exogenously added AA (6 fold in the presence and 18 fold in the absence of AA). Furthermore, cells from passage 15 are far more susceptible to A23187 stimulation in the endogenous PGE_2 synthesis (528% over control) than that from passage 93 (200% over control).

Proposed Course:

Our preliminary results demonstrate that the alteration of biochemical properties in PG biosynthesis occurred during continuous cell subculture. Concomitant with this study, the morphology of this cell line was found to change from LCC (initial tumor) to small cell carcinoma which exhibits endocrine cell features (passage 15) and subsequently to oncocytoma (passage 93, containing large numbers of mitochondria). The existence of such a unique cell line provides us an opportunity to study the interrelationship of AA metabolism and the pathobiology of lung carcinoma. Further *in vitro* studies are planned to

evaluate the lipoxygenase pathways of AA metabolism in these cells. Homogenous cells of NCI-H460 with early as well as late passages will be injected into nude mice, the profile of AA metabolism and degree of tumor development will be determined. Inhibitors of AA metabolism such as indomethacin, aspirin, flufenamic acid and nordihydroguaiaretic acid will be administered before or at different times after injection of the tumor cells from different passages. The effect of these compounds on the overall metabolism of AA and tumor and/or metastases formation will be evaluated. Moreover, additional representative cell lines derived from large cell carcinomas will be investigated. The above proposed studies together with those proposed in Annual Report Z01 CM 07173-01 LETM will allow us to better assess the role of AA metabolism in this heterogenous class of human lung cancer.

Significance to Biomedical Research and the Program of the Institute:

It is clear that a selective therapeutic attack on cancer cells is possible only when the basic differences between cancer and normal cells are well understood. The PGs and related eicosanoids have been implicated as important factors in a number of pathobiological aspects of malignant disease. In addition, they have been implicated as mediators in certain aspects of metastatic dissemination of human primary tumors. Due to the fact that pulmonary LCC is the least treatable lung cancer, investigations of the biochemical function of LCC is warranted. The proposed studies should allow us to evaluate the role of AA in tumor differentiation and/or metastases formation in experimental animals after injection of the homogenous cells from different passages, especially those from the initial passage. Such investigation should also allow us to determine whether or not the profile of AA metabolism in human lung cells *in vitro* accurately reflects AA metabolism *in vivo* in different stages of the disease.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07152-02 LETM

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Studies on Clara cell mediated lung carcinogenesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. M. Schuller	Vet. Med. Officer	LETM, NCI
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COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	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.)*

A number of N-nitrosamines are powerful respiratory tract carcinogens which require metabolic activation in the host organism. This metabolic activation is believed to be mediated by cytochrome P-450 enzymes although unequivocal evidence for this hypothesis has not yet been achieved. An experiment was conducted to investigate the effect of the P-450 enzyme inhibitor, piperonyl butoxide, on the induction of lung tumors which originate from Clara cells in hamsters treated with N-nitrosodiethylamine (DEN). The effect of piperonyl butoxide on covalent binding and distribution of the parent nitrosamine was examined in vivo after 1 dose of ^{14}C-DEN. Moreover, the effect of piperonyl butoxide on the tumor incidence induced by DEN in a chronic study was investigated by histopathology. Piperonyl butoxide significantly inhibited metabolism of DEN in the respiratory tract and inhibited the induction of lung tumors. These data provide the first experimental evidence for cytochrome P-450 enzymes being a crucial factor to the metabolic conversion of DEN into a carcinogenic metabolite in vivo.

As a comparative approach, human lung cancer cell lines (HCCL) with and without morphological features of pulmonary Clara cells were tested for their ability to metabolize DEN in vitro. It was found that the cell line with Clara cell features metabolized DEN to a significantly greater extent than cell lines derived from other types of lung cancers and that such metabolism was significantly inhibited by cytochrome P-450 inhibitors. Moreover, an HCCL with morphological features of alveolar type II cells was found to metabolize DEN to a great extent via prostaglandin endoperoxide synthetase. This is the first experimental evidence for different cell types in the lung to metabolize a nitrosamine via different pathways and may be of potentially great importance in explaining the cell type-specific effects of nitrosamines.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives:

N-nitrosamines are ubiquitous in man's environment and are formed endogenously by reaction between amine precursors and nitrosating agents. They require metabolic activation in the host organism which is believed to be mediated by cytochrome P-450 enzymes. However, proof for this hypothesis has not yet been reached. N-nitrosodiethylamine (DEN) is a powerful respiratory tract carcinogen in hamsters. Studies on the pathogenesis of the lung tumors and autoradiographic studies on the site of covalent binding in the lungs have given evidence for the pulmonary Clara cell as being the origin of lung tumors and an important site of covalent binding in the lungs. The objective of this study was to examine if cytochrome P-450 enzymes are an important factor for the activation of DEN to carcinogenic metabolites in this in vivo system and if parallel data could be obtained using well-differentiated human lung cancer cell lines of defined cell type.

Methods Employed:

In vivo studies. Initial studies were undertaken to determine the time of maximum covalent binding in trachea, lung and liver after injection of ^{14}C-DEN. The time-point of maximum covalent binding (4 hr) was then selected to study the effect of pretreatment with the cytochrome P-450 inhibitor, piperonyl butoxide, on covalent binding as well as distribution of ^{14}C-DEN. In another experiment, hamsters were s.c. injected 2 x per week for 20 weeks with DEN while others received pretreatment with piperonyl butoxide before each DEN injection. One group injected with piperonyl butoxide alone, and one injected with saline (vehicle of DEN) served as controls. All animals were sacrificed after 20 weeks on test at which time DEN alone had induced tumors in lung and trachea in about 70% of the animals as shown by earlier experiments. Complete autopsies were done on all animals, and lungs and livers were processed for histopathology from paraffin sections stained with hematoxylin/eosin and PAS-reaction. Tissue samples of these organs were also processed for electron microscopic investigation.

In vitro studies. The metabolism of ^{14}C-DEN was studied in the following cell lines: NCI-H322 derived from human pulmonary adenocarcinoma and exhibiting ultrastructural features of Clara cells; NCI-H358 derived from human pulmonary adenocarcinoma and exhibiting ultrastructural features of alveolar type II cells; NCI-H128 and NCI-H69 both derived from human pulmonary small cell cancer and demonstrating ultrastructural features of poorly differentiated endocrine cells. Metabolism of the nitrosamine was monitored by $^{14}CO_2$ production and covalent binding of radiolabel from ^{14}C-DEN to cell proteins and DNA. The effect of pre-exposure to nitrogen or a carbon monoxide/oxygen mixture on such metabolism was studied as was the effect of preincubation with aspirin and indomethacin.

Major Findings:

In the hamster model in vivo, pretreatment with piperonyl butoxide 2 hrs before injection of ^{14}C-DEN resulted in a 90% inhibition of covalent binding in lung and liver. In the chronic experiment, 9 out of 10 hamsters treated with DEN alone had multiple tumors in the lungs. In the animals pretreated with piperonyl butoxide, no tumors developed in the lungs.

Human lung cancer cell lines in vitro. ^{14}C-DEN was metabolized by adenocarcinoma-derived NCI-H322 (with Clara cell features) to a significantly greater extent than by NCI-H358 (with features of alveolar type II cells) while NCI-H69 and NCI-H128 (derived from small cell carcinoma) were unable to metabolize the compound. Metabolism was markedly inhibited by heat denaturation of the cell protein. ^{14}C-DEN metabolism by NCI-H322 was greatly decreased when the incubation was carried out under (i) anaerobic conditions and (ii) in the presence of a carbon monoxide enriched atmosphere. These results suggested the involvement of a cytochrome P-450-dependent monooxygenase enzyme system. Metabolism by NCI-H358 was also decreased in the absence of oxygen or presence of carbon monoxide although the effects were relatively small compared to the results with NCI-H322. On the other hand, aspirin or indomethacin, which are inhibitors of the fatty acid cyclooxygenase component of prostaglandin endoperoxide synthetase, preferentially inhibited ^{14}C-DEN metabolism by NCI-H358. There were little or no effects of these inhibitors on the metabolism of DEN in NCI-H322. The data suggest that DEN metabolism in different lung cell types may be carried out by different enzyme systems which in turn may contribute to the selective effect of DEN in the lung.

Significance to Biomedical Research and the Program of the Institute:

The identification of in vivo mediators of respiratory tract carcinogenesis induced by xenobiotics is an important step towards an understanding of the mechanism of carcinogenesis in this organ system. However, to extrapolate such data generated in an animal model to man, it is vital to obtain comparative data using the respective human organs and cell types. In the absence of isolated systems of normal human cell types, well differentiated early passage lung cancer cell lines comprised of one clearly identifiable cell type are a valuable tool for this approach. Such knowledge is an important basis for the development of target oriented inhibition and treatment of respiratory tract cancer which may lead to the development of anticancer drugs with high target specificity.

Proposed Course:

Further in vivo studies will focus on the comparison of ultrastructural changes induced in this model system during DEN carcinogenesis with and without pretreatment with piperonyl butoxide. Also, autoradiographic studies will be applied to investigate the effect of piperonyl butoxide on the distribution of bound radioactivity in the respiratory organs after injection of ^{14}C-DEN. Moreover, comparative studies with inhibitors of other enzyme systems will be conducted. The planned complementary in vitro studies include the identification of DNA adducts of DEN-exposed HCCL as well as identification of the metabolites formed. Moreover, isolated rat and hamster lung cell types will be used for the same assays.

Publications:

1. Reznik-Schüller, H.M.: Experimental carcinogenesis of bronchiolo-alveolar neoplasms. In McDowell, E.M. (Ed.): Current Problems in Tumor Pathology. Vol. III. Lung Tumor Pathobiology. Edinburgh, Churchill-Livingston, in press.

2. Pour, P. and Reznik-Schüller, H.M.: Squamous cell carcinomas of the bronchi in Syrian golden hamsters. In Jones, T.C. and Mohr, U. (Eds.): ILSI Monographs on Pathology of Laboratory Animals. Vol. 2, Respiratory System, in press.

3. Schuller, H.M., McMahon, J.B.: Inhibition of N-nitrosodiethylamine-induced respiratory tract carcinogenesis by piperonyl butoxide in hamsters. Cancer Res., in press.

4. Falzon, M., McMahon, J.B., Gazdar, A.F., and Schuller, H.M.: Preferential metabolism of N-nitrosodiethylamine by two cell lines derived from human pulmonary adenocarcinomas. Carcinogenesis, in press.

5. Schuller, H.M., Stinson, S.F., Ward, J.M., McMahon, J.B., Singh, G., Katayl, S.L.: Loss of Clara cell antigens from neoplastic Clara cell derived lesions induced in the hamster lung by N-nitrosodiethylamine. Am. J. Pathol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07153-02 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Biology of Human Lung Cancer Cell Lines In Vitro

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. B. McMahon	Cancer Expert	LETM, NCI
Others:	M. Falzon	Visiting Fellow	LETM, NCI
	H. M. Schuller	Vet. Med. Officer	LETM, NCI
	A. C. Smith	Staff Fellow	LETM, NCI
	A. A. del Campo	Bio. Lab. Tech.	LETM, NCI
	M. R. Boyd	Associate Director	DTP, NCI

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	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.)*

Lung cancer is one of the most common, most lethal, but least treatable diseases. All currently available anticancer drugs are essentially ineffective against most human lung cancers. For the purpose of therapy, lung cancers are generally classified into small cell and non-small cell cancers. With respect to the many different tumor types found in the category of non-small cell cancer, it is difficult to imagine that any one anticancer drug can be effective against all of them. This project utilizes the subclassification of cell lines by cell type and differentiation generated by Annual Report Z01 CM 07175-01 LETM to further define biological and functional aspects of these defined cell lines in vitro. Such studies include scanning and transmission electron microscopy, quantitative image analysis, detailed analysis of cytoplasmic enzymes, antigenic expression, endocrine and/or secretory activity, as well as competence for binding and metabolism of 4-ipomeanol and other candidate anticancer drugs. We found clear cut cell type specific differences for almost every parameter studied. Moreover, we found that some of the well differentiated tumor cell lines have retained biochemical pathways which are considered functional markers of their normal cells of origin. Among those were: expression of monooxygenase and other xenobiotic-metabolizing enzyme activities (markers of normal pulmonary Clara cells) in an adenocarcinoma line with the ultrastructure of Clara cells; phospholipid biosynthesis (marker for normal alveolar type II cells) in an adenocarcinoma line with ultrastructure of alveolar type II cells; neuroendocrine activity in several small cell cancer lines and carcinoid lines. We also found cell type specific susceptibility to drug-induced toxicity, selectively high levels of prostaglandin synthetase activity which were cell type and differentiation dependent in collaboration with other investigators within the LETM.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

Although human lung cancer is usually divided into two broad histopathological categories, namely small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), these tumors are far more complex. Often human tumor lines are comprised of a mixture of functionally and morphologically different cell types. In order to effectively design therapy for these tumors, the cell biology of the individual cell types must be studied in detail. The availability of a large number of human lung cancer cell lines derived from tumors of specific cell types may provide a means to this end. By carefully characterizing biological, functional and pharmacological aspects of cell lines of defined cell type and differentiation (generated by ultrastructural analysis; see Annual Report Z01 CM 07175-01 LETM), we may be able to discover individual traits expressed by different tumor cell types which will allow us to tailor therapy.

Methods Employed:

Human lung cancer cell lines were obtained from Dr. Adi Gazdar, NCI-Navy Clinical Oncology Branch. The culture conditions varied according to the growth characterization of the cells. SCLC cell lines and carcinoids were grown as suspension cultures while NSCLC cell lines were maintained as monolayer cultures. All cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. All cell lines and tumor xenographs were diagnosed at the ultrastructural level by Dr. H.M. Schuller upon arrival at LETM (see Annual Report Z01 CM 07175-01 LETM). Further ultrastructural analysis under various experimental conditions was done with an analytical electron microscope using the transmission mode at 80 KV. SCLC cell lines and carcinoids were analyzed for amine precursor uptake and decarboxylation (APUD) features such as formaldehyde-induced fluorescence (FIF); L-dopadecarboxylase (DDC) activity; cytoplasmic dense-cored granules; production of the polypeptide hormone, calcitonin and bombesin, and intracellular 5-HT content assessed by immunoperoxidase staining. These cell lines were also analyzed for the modulation of these APUD cell features by biogenic amines. The ability of the cell lines to produce prostglandins from arachidonic acid as well as to use arachidonic acid as a substrate for cooxidation reactions was carried out as part of a collaborative project with Dr. S. Lau (see Annual Report Z01 CM 07173-01 LETM). In order to assess whether the lung cancer cell lines possessed the metabolic machinery known to exist in specific normal lung cell types a variety of assays were performed. 4-Ipomeanol, a candidate anticancer drug with known target cell specificity toward normal rodent Clara cells, was used as a model substrate for cytochrome P-450 activity (a marker of pulmonary Clara cells). The metabolism of 4-ipomeanol was monitored by covalent binding of labeled 4-ipomeanol. Known metabolic inhibitors and inducers were used along with these assays. Assays for specific enzymes involved in xenobiotic metabolism were carried out using standard techniques modified for cultured cells. The cytotoxicity of 4-ipomeanol as well as other candidate anticancer drugs. was monitored by colony forming assays and soft agar clonigenic assay systems. Analysis of phospholipid biosynthesis by the cell lines was monitored by ^{14}C-choline incorporation into cellular lipids and also secreted into the extracellular media.

Major Findings:

When the human lung cancer cell lines were analyzed for a wide variety of properties, the following results and observations were found:

1. Human lung carcinoid cell lines and SCLC lines had both retained a number of functional properties which are the markers of their cell types of origin (pulmonary endocrine cell). These included induction of dense-cored granules by 5-HTP, kinetics of FIF expression, and production of polypeptide hormones.

2. Some well differentiated cell lines, both of the SCLC and NSCLC types were able to synthesize prostaglandins (see Annual Report Z01 CM 07173-01 LETM).

3. The toxicity of Ara-AC (see Annual Report Z01 CM 07122-05 LPET) to a panel of NSLC cell lines correlated with the cellular deoxycytidine kinase activity.

4. Two NSCLC cell lines NCI-H322 and NCI-H358, which retain many properties of Clara cells and alveolar type II cells respectively, possess cytochrome P_{448} dependent monooxygenase activity. Furthermore, other microsomal enzymes such as cytochrome b_5, NADPH cytochrome c reductase and epoxide hydrolase are also expressed.

5. The metabolism-dependent covalent binding of 4-ipomeanol to cellular proteins was evident only in bronchiolo-alveolar carcinoma derived cell lines and was absent in the SCLC cell lines. The metabolism to the reactive intermediate was oxygen dependent, inducible, substrate concentration and time-dependent.

6. Cytotoxicity of 4-ipomeanol to NSCLC cell lines was greater in the cell line with Clara cell features than in other cell lines.

7. The NCI-H358 cell line which has characteristics of alveolar type II cells had retained the marker function of its cell of origin to convert choline into phosphatidyl choline, an essential component of lung surfactant.

Significance to Biomedical Research and the Program of the Institute:

Lung cancer in man presents vast problems to the scientist both from a clinical standpoint and from basic cell biology. Therapeutic responses are difficult to predict in part because most human lung tumors are heterogeneous and often contain malignant cells of different functional and morphological types. Although recent studies on the various cell types in mammalian lung during normal differentiation and in response to injury have added much to our knowledge, the cellular mechanism responsible for the generation of heterogeneity and the interconversions of cell types seen in malignancy are poorly understood. Detailed studies on the cell biology of human cancer cell lines under controlled in vitro conditions may aid in our understanding of these complex processes and help design more appropriate therapies. Moreover, well differentiated cell lines at early passages represent a valuable tool to study cell-type specific biology, biochemistry and pharmacology of identified lung cell types. In the absence of in vitro systems to study individual normal types of lung cells, such data will much improve our understanding of functional aspects mediated by cell type in the normal lung.

Proposed Course:

The acquisition and characterization of more human cell lines of specific cell types is the key component in the proposed future course of this project. We plan to use as many early passage cell lines as possible established from human tumors which have been diagnosed and characterized at the electron microscope level (see Annual Report Z01 CM 07175-01 LETM). In this way we can more accurately correlate our observations in vitro with those encountered in vivo. Studies on the chemical and biochemical properties of these cell lines will be expanded both in our laboratory as well as through collaborative projects inside and outside of NIH. This project will work closely with the LCDDP, as well as the new DCT lung cancer drug discovery project, in order to provide some basic science support for the mechanisms of action of newly acquired cancer chemotherapeutic agents.

Publication:

1. McMahon, J.B., Reznik-Schüller, H.M., Gazdar, A.F. and Becker, K.L.: Influence of priming with 5-hydroxytryptophan on APUD-characteristics in human small cell lung cancer cell lines. Lung 162: 261-269, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07154-02 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*: Isolation, Characterization & Selective Growth of Rodent Lung Cell Types In Vitro

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, leboretory, and institute affiliation)*

PI:	J. B. McMahon	Cancer Expert	LETM, NCI
Others:	A. C. Smith	Staff Fellow	LETM, NCI
	A. A. del Campo	Bio. Lab. Tech.	LETM, NCI
	D. Gronski	Bio. Lab. Aid	LETM, NCI
	H. M. Schuller	Vet. Med. Officer	LETM, NCI

COOPERATING UNITS *(if eny)*:

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the spece provided.)*

It is well established that certain pulmonary toxins and carcinogens act on specific cell types of the rodent lungs. Metabolism studies in vivo, or experiments using whole organ homogenates and fractions, may therefore not be suited to detect metabolic pathways operative in such specific cell types. It is the objective of this project to isolate and selectively grow the major epithelial cell types of the rat and hamster lung for comparative studies on their biology and response to toxins, carcinogens and anticancer drugs. These defined cell populations will be used for comparative studies on the effects of various pulmonary agents on human lung cancer cell lines (see Annual Report Z01 CM 07152-02 LETM). Immunoperoxidase staining of isolated rat lung epithelial cells with an antibody preparation specific for rat surfactant apoproteins has revealed that rat alveolar type II cells can be purified to 95% by selective plating and proliferation. These cells which proliferated in vitro were shown to retain their epithelial morphology and contained osmiophilic bodies for 5 days in culture. This specific antisera preparation revealed that the isolated type II cells retained the capability to produce surfactant-like material. Using immunoperoxidase method, two patterns of staining were observed, one being a diffuse cytoplasmic stain and the other consisting of intensely stained cytoplasmic structures. These different patterns may reflect different stages of maturation of lamellar structures during the production and secretion of lung surfactant apoprotein. Analysis of phospholipid biosynthesis revealed that the isolated alveolar type II cells actively converted choline into phosphatidylcholine and that the prosynthetic activity increased with time in culture. The morphology of the isolated type II cells as well as other isolated cell types was characterized by a newly established technique which allows for correlative morphological characterization by light and electron microscopy.

Objectives:

Many pulmonary toxins and carcinogens act on specific cell types in the lung. These cells are capable of activating potentially toxic or carcinogenic compounds present in the circulation and thus can mediate the toxic and/or carcinogenic responses. Due to the heterogeneous nature of the lung, investigations on the metabolic activation of endogenous and exogenous substrates by specific cell types in vivo have often been difficult to interpret. The objectives of this study are to develop methods for the isolation and purification of specific cell types from the lungs of animals that have known susceptibility to certain toxins and carcinogens. Modification of existing technologies such as immunocytochemistry and correlative light and electron microscopy are being utilized to clearly identify the isolated cell populations. Once established, these defined cell populations will allow the investigation of the biochemical mechanisms of selected compounds at a cellular and subcellular level. The principal cells of interest include alveolar type II cells, Clara cells and endocrine cells, and in rat and hamster lungs. Compounds of known in vivo specificity for certain cell types such as BCNU, 4-ipomeanol and diethylnitrosamine are initially studied in these in vitro systems in order to establish if the isolated cells are functionally comparable with their counterparts in vivo.

Methods Employed:

Lung cell types are isolated using both published as well as newly developed techniques. Rat and hamster alveolar type II cells are isolated by intratracheal elastase digestion. Macrophages from both species are isolated by lavage and primary adherence. Interstitial fibroblasts are isolated by collagenase digestion of lung tissue that had been pretreated with elastase to remove the epithelial cell component. Clara cells are purified from elastase treated lungs by differential plating and density gradient centrifugation. A variety of techniques are used to identify and quantitate the isolated lung cell preparations. These include the use of antibody preparations obtained through a collaborative effort with Dr. Singh at the University of Pittsburg School fo Medicine VA Medical Center. One of these immunological reagents is specific for the surfactant apoprotein secreted by type II cells while another reacts specifically with secretory proteins of Clara cells. Immunological staining utilizes the peroxidase antiperoxidase method using diaminobenzodine as the indicator. The metabolism of 4-ipomeanol was monitored by covalent binding to cell macromolecules. Phospholipid biosynthesis was monitored by the incorporation of ^{14}C-choline into cellular and secreted phosphatidylcholine. Correlative light and electron microscopy were done as follows: Cells were grown on Aclar film (Allied Chemical) on which a locator grid was drawn with India ink. After photomicroscopical documentation of colonies of interest under the inverted photomicroscope, the same film with the attached cells is processed for transmission electron microscopy. The resulting EPON replicas are stained with Toluidine Blue for high resolution bright field light microscopy, allowing the precise localization of specific cell colonies previously photographed. These areas are then trimmed and thin sectioned for further evaluation by transmission electron microscopy.

Major Findings:

Using isolated lung cell preparations under various experimental conditions, the following results and observations were found:

1. Alveolar type II cells can be isolated from rat lung with a viability of 96 ± 3% (n=20). After primary plating (24 hr), 75% of the attached cells react with the anti-rat surfactant apoprotein antiserum, the attached cells proliferate for approximately 7 days and by the 4th day, 94% of the cells present are type II cells as shown by both immunoreactivity and electron microscopy.

2. The isolated rat alveolar type II cells incorporated ^{14}C-choline into cellular phosphatidylcholine (PC) and some of the ^{14}C-PC was secreted into the media. There was a 200% increase in PC biosynthesis after 48 hr in culture and a 20% increase after 72 hr while the secretion rate of ^{14}C-PC remained constant for 72 hr in culture.

3. The metabolic activation of ^{3}H-ipomeanol was studied in vitro in isolated lung cell preparation from rats and hamsters. Covalent binding of ^{3}H-ipomeanol was significantly higher in hamster lung cells than in rat lung cells. The binding in both species was found to be dependent on the cytochrome P-450 monooxygenase system.

Significance to Biomedical Research and the Program of the Institute:

The development of procedures for the isolation and characterization of specific lung cell types is essential for the study of the mechanisms of toxicity and carcinogenesis involving epithelial cell types of the lungs. Specific lung cell populations isolated from animals of various species may provide insight into the species and tissue specificities often displayed by pulmonary toxins and carcinogens. Manipulating the cells under controlled conditions may allow for the development of strategies to intervene in the toxic or carcinogenesis process. Preparations of isolated rodent lung cell types which have been recognized as targets of xenobiotics through in vivo experimentation present a useful tool to elucidate critical cell type-specific pathways responsible for the selective effects. A typical example for this approach is presented in a collaborative study (see Annual Report Z01 CM 07140-03 LETM) which reports the effect of BCNU on rat alveolar type II cells in vitro, a model system for BCNU-induced fibrosis in man.

Proposed Course:

The future efforts of this project will include improving the techniques for lung cell isolation and identification. A large portion of the efforts will be devoted to the purification of hamster and rat Clara cells and APUD cells and methods to clearly identify these cell types in vitro. Comparative studies will be undertaken to examine the possible causes for the species and tissue specificities of model compounds such as BCNU, DEN and 4-ipomeanol. Specific immunological reagents will be used in future studies for characterization and identification especially with respect to Clara cells and type II cells. Another important direction for this project involves the development of methods

to facilitate the morphological characterization of the isolated lung cell preparations. This is critical in that techniques that attempt to isolate specific cell types from organ systems, i.e., lung, depend on reliable methods of ultrastructural identification in addition to biochemical and/or histochemical investigations.

Therefore, we will develop correlative techniques that will combine the convenience and large sampling capability of light microscopy with ultrastructure evaluation to aid in the identification of particular cell types.

Publication:

1. McMahon, J.B., Smith, A.C., del Campo, A.A., Sing, G., Katyal, S., and Schuller, H.M.: Characterization of proliferating rat alveolar type II cells in vitro by immunological biochemical and morphological criteria. Exp. Lung Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07175-01 LETM

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Ultrastructural Classification of Human Lung Cancers and Cell Lines

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	H. M. Schuller	Vet. Med. Officer	LETM, NCI
Others:	A. A. del Campo	Bio. Lab. Tech.	LETM, NCI
	M. Gregg	Chemist	LETM, NCI
	S. Walton	Bio. Lab. Aid	LETM, NCI

COOPERATING UNITS *(if any)*: Clinical Oncology Branch, NCI-Navy (A. F. Gazdar)

LAB/BRANCH: Laboratory of Experimental Therapeutics and Metabolism

SECTION: Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	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 histopathology classification of lung cancers and cell lines is based on criteria detectable by light microscopy such as cell size and shape as well as growth patterns. Attempts to characterize the tumors by cell type are generally beyond the resolution of light microscopy. This is the reason why tumors of a given histopathology type may be comprised of a large variety of different cell types and of mixtures of cells. The reaction of cells and tissues including tumors to chemicals and drugs is largely dictated by their biochemical machinery which in turn is cell type specific. Efforts to study the response of lung tumors and cell lines to chemicals and drugs require, therefore, that the tumors be classified by cell type and differentiation using electron microscopy.

We therefore investigate surgical samples of lung cancers as well as the cell lines derived from them by electron microscopy and classify them by cell type and differentiation. Since it is presently not known to what extent the HCCL change during prolonged life in vitro, we also monitor the cell lines at early and late passages. To date, we have processed 31 surgical samples which are currently being diagnosed. A total of 25 cell lines has thus far been investigated which resulted in modified or altered diagnosis in many cases. Some of the lines were examined at early and late passages and there is evidence that, in particular, well differentiated tumors tend to change morphologically after several passages in vitro. We are using the ultrastructural classification of tumors and cell lines as a basis for the design of comparative biochemical and pharmacological experiments (within LETM and in collaboration with other institutes) aimed at elucidating cell type specific reactions.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives:

The histopathology diagnosis of lung cancers and cell lines derived from them is insuficient in that it does not allow for a classification by cell type and differentiation. However, the response of cells including tumor cells is largely mediated by their biochemical machinery which in turn is cell type and differentiation dependent. We therefore subclassify surgical samples of human lung tumors and cell lines derived from them by electron microscopy to provide this vital information. The ultrastructural classification is then used as a basis to select tumors and cell lines for experiments on biology, metabolic competence and response to drugs (including anticancer drugs). It is anticipated that by comparing such data derived from material of known cell type and degree of differentiation cell type-specific behavior and reactions will be detected. This information is vital for all research within LETM on various biological and biochemical aspects of lung cancers and cell lines. Moreover, these data represent a much needed asset for the interdisciplinary DCT lung cancer drug discovery project which is using the very same cell lines for drug screening.

Methods Employed:

Surgical samples (supplied by the NCI-Navy Clinical Oncology Branch, Dr. Gazdar) are processed for high resolution light-microscopy and transmission electron microscopy according to standard procedures. All samples are divided into osmicated and non-osmicated material to allow for immunocytochemistry and/or special stains, if required. Cell lines are processed similarly with slight modifications to enhance the contrast. All samples (surgical and cell lines) are diagnosed histopathologically by high resolution light microscopy and photomicrographs of representative areas are taken for recording. Such representative areas are then thin sectioned for diagnosis at the electron microscopic level. EM diagnosing and photography are done using a JEOL 100 CX electron microscope operated at 60 KV. The ultrastructural morphology of surgical samples is compared with the cell lines derived from them as are early and late passages of individual cell lines.

Major Findings:

Of the surgical samples investigated to date, one bronchiolo-alveolar carcinoma was subclassified as an endocrine tumor due to the presence of dense-cored endocrine-type cytoplasmic granules. In another case, a large cell carcinoma was reclassified as oncocytoma with possible steroid hormone production because of abundant mitochondria with tubular christae. Of the 25 cell lines studied to date, seven resulted in an ultrastructural diagnosis different from the original histopathology diagnosis of their tumors of origin. Moreover, ultrastructural classification added the subclassification of adenocarcinomas by cell type which lead to the identification of a line with features of pulmonary Clara cells (NCI-H322) and alveolar type II cells (NCI-H358), respectively, which were used for studies on metabolism of diethylnitrosamine and 4-ipomeanol as well as phospholipid synthesis and prostaglandin synthetase activity in collaboration with other investigators within LETM and within PUOS.

Studies of early and late passages of a cell line (NCI-H460) derived from a large cell carcinoma resulted in reclassification of the early passage into small cell cancer because of the presence of dense-cored endocrine-type granules and demonstrated the gradual transition of such cells into oncocytes in the later passages. Electron microscopic diagnosis of early passages - derived from four suspected carcinoid-tumors confirmed the tentative histopathology diagnosis and resulted in the identification of one unique well differentiated carcinoid cell line (NCI-H727) with features of normal pulmonary endocrine cells. This cell line is of much interest to study the physiological role of normal pulmonary endocrine cells and of their reaction to chemicals and drugs.

Significance to Biomedical Research and the Program of the Institute:

The outlined ultrastructural classification of lung cancers and cell lines by cell type and differentiation is the vital basis for all ongoing and future research on lung cancer biology and therapy within LETM and to the DCT lung cancer drug discovery project. It makes it possible to detect and compare cell type and differentiation dependent biological, biochemical and pharmacological properties. Such data will substantially further our understanding of functional aspects of normal cell types and of the cancers derived from them and will pave the way towards a disease-oriented "custom-tailored" cancer therapy.

Proposed Course:

This is a long-term project that will have to run over several years in order to collect statistically significant data. While during the current initial period the major emphasis is on diagnostic aspects, future studies will also include electron microscopic investigations on the subcellular effects of chemicals and drugs. Such data are anticipated to generate insight into intracellular targets at the organelle level which can then be used for experiments focused on biochemical mechanisms of chemicals and drugs.

Publication:

1. Schuller, H.M., del Campo, A., McMahon, J.B., Oie, H.B., Boyd, M.R. and Gazdar, A.F.: Ultrastructural classification of human lung cancer cell lines. JNCI, in press.

ANNUAL REPORT OF THE LABORATORY OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

The Laboratory of Pharmacology and Experimental Therapeutics (formerly 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 organizational components are the Medicinal Chemistry Section, concerned with the rational design and synthesis of new agents, and the Biochemical Pharmacology Section, concerned primarily with fundamental mode of action studies. In addition, the Laboratory as a whole develops analytical methodology for the detection of antitumor drugs and their metabolites, and also participates actively in the clinical pharmacology/Phase I trials of new agents which either originated within the Laboratory or which were derived from extramural sources but in whose preclinical development the Laboratory has played a major role.

During the past year, the design and synthesis of potential differentiating agents has continued within the Medicinal Chemistry Section of the Laboratory. At least 12 recently synthesized compounds have proven to be as active as 1-methyl-2-pyridone (MP), the prototype reference compound, while several from among this group have been found to be significantly more potent than MP in terms of differentiating activity, utilizing the HL-60 test system. The most active compound found to date is a cyclopropyl analog of 2-pyridone.

We have recently shown that arabinosyl-5-azacytosine (ara-5-AC), a compound originally synthesized within LPET and presently under active development toward clinical trial, exhibits unexpectedly high anticerebral antitumor activity in the IC L1210 murine test system (i.e., activity greater than that of ara-C, and comparable to that of the prototype reference compound, BCNU). In addition, we have recently developed a rapid and sensitive HPLC assay for the quantitation of ara-5-AC in biological fluids. These findings will be of direct applicability in the projected Phase I/II clinical trials of this compound shortly to be initiated by DCT.

The LPET compound, 2-oxypyrimidine riboside (zebularine), shows significant, although not outstanding, activity in the L1210 and B16 melanoma test systems. We have recently found this compound to exhibit unusually high activity against both DNA virus (herpes simplex and adenovirus) and RNA virus (poliovirus Type III and dengue virus) test systems. The mechanism for this unanticipated activity is now being explored.

Analytical methodology has been developed for the determination of the LPET-developed compound spirohydantoin mustard (spiromustine), and clinical pharmacology studies are being actively pursued in collaboration with COP, DCT, with NINCDS, NIH and also with extramural institutions participating in the Phase I trial of this agent.

In last year's Annual Report, a total synthesis of the antitumor antibiotic Neplanocin A was described. This synthetic scheme has now been shortened and improved, and further adapted for utilization in the synthesis of other purine and pyrimidine cyclopentenyl nucleoside analogs of the neplanocin class. This series is of interest because of the antitumor activity of many of these compounds, and also because of their other biological properties such as antiviral activity and inhibition of the enzyme uridine-cytidine kinase.

In another area of synthesis, all the possible phosphonate analogs of the active metabolite of TAD (the active anabolite of tiazofurin) have now been prepared. One of these (β-methylene TAD) was equipotent to TAD as an IMPD inhibitor and possesses the advantage of being resistant toward cleavage by TAD-inactivating enzymes.

In studies of differences between metastatic cells and their non-metastatic counterparts, an L1210 system utilizing tumor cells isolated from murine liver has been utilized. Marked differences have been observed between liver metastatic L1210 tumor cells and their ascitic counterparts, e.g., a 2-fold increase in glutathione concentration and a 5-fold greater level of surface SH-groups in the liver metastatic cells. The metastatic cells are more resistant to melphalan, but can be resensitized by blocking the surface sulfhydryls with 6,6-dithionicotinic acid.

Studies have continued within LPET on the process of tubulin assembly and on the effect of known and potential antimitotic drugs on this process. One of the "microtubule-associated proteins" (MAPS), has now been purified to homogeneity; this protein causes the formation of microtubule "bundles" (distinct microtubules which aggregate laterally). The active component adheres to hydroxylapatite but not to DEAE-cellulose, is heat stable, and can be reconstituted after denaturation in 8 M urea. The fraction has now been purified by preparative HPLC, resulting in an electrophoretically homogeneous protein made up of two subunits with molecular weights ca. 25,000. Extensive studies on new tubulin inhibitors (combretastatin, 6-benzyl-1,3-benzodioxole derivatives, 5,6-diarylpyridazin-3-one derivatives) have also been initiated, with a view to establishing their site and mechanism of action at the molecular level.

In the area of drug resistance, studies have been carried out on both natural and acquired resistance to ara-5-AC, an LPET compound soon to enter Phase I clinical trial. In most test systems, resistance was most commonly accompanied by a decreased level or in some cases, by a complete deletion of deoxycytidine kinase, the enzyme responsible for anabolic activation of the drug. In terms of catabolism, ara-5-AC, unlike ara-C, is not inactivated by mammalian cytidine deaminase, an observation which may be of relevance in initial clinical dose-scheduling of the drug.

The new agent, merbarone (NSC 336629), has been extensively examined in terms of possible mechanism of action. The compound is an effective but atypical inhibitor of DNA synthesis; among a number of factors examined, this inhibition appears to correlate most closely with the ability of the compound to generate single-strand DNA breaks which do not, however, appear to be protein-associated. Investigation of the molecular pharmacology of this unusual wide-spectrum antitumor agent is continuing.

In conjunction with its research output in fields related to drug development, the Laboratory of Pharmacology and Experimental Therapeutics continued its active publication record in 1984-85. A total of 52 papers describing these and related studies appeared or were accepted for publication during the current year; these publications are listed in the following section of this report.

Publications:

1. Batra, J.K., Jurd, L. and Hamel, E.: Structure-function studies with derivatives of 6-benzyl-1,3-benzodiazole, a new class of synthetic compounds which inhibit tubulin polymerization and mitosis. Mol. Pharmacol., 27: 94-102, 1985.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03580-16 LPET

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. S. Driscoll	Head, Medicinal Chemistry Section	LPET, NCI
Others:	V. E. Marquez	Visiting Scientist	LPET, NCI
	A. Haces	Visiting Fellow	LPET, NCI

COOPERATING UNITS *(if any)*

Laboratory of Biological Chemistry, Drug Evaluation Branch

LAB/BRANCH

Laboratory of Pharmacology and Experimental Therapeutics

SECTION

Medicinal Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.7	1.7	

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.)*

The objective of this work is the discovery of new drugs which are clinically useful against cancer. The following topics are of current interest: 1) synthesis and evaluation of differentiating agents, 2) plasma stability of spiromustine analogs, 3) ara-AC intracerebral antitumor activity, 4) antitumor, antiviral and cytidine deaminase inhibition of zebularine and 5) potentiation of AZQ CNS antitumor activity.

PHS 6040 (Rev. 1/84) GPO 914-918

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. Synthesis of differentiating agents
2. Synthesis of spiromustine analogs
3. Ara-AC activity against IC L1210 leukemia
4. Activity of Zebularine
5. Potentiation of AZQ CNS antitumor activity

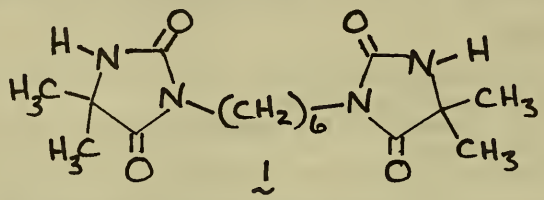
Synthesis of Differentiating Agents (Drs. Haces, Breitman, Driscoll). A number of chemicals have been shown to cause certain murine or human tumor cells to differentiate into more mature cells with reduced proliferative properties. The mechanism of action of these materials is unknown. Hexamethylene bisacetamide (HMBA) is one of these chemicals is presently the subject of an NCI sponsored Phase I clinical trial. It now appears that the concentration of HMBA which can be attained in humans may be marginal with respect to the concentrations anticipated necessary for differentiation. Therefore, new differentiating agents with higher potencies are required. Structure-activity studies were initiated in two series of compounds known to possess differentiating properties - the bisamides and the pyridones.

HMBA Analogs. Shortly after the discovery of the differentiating properties of HMBA, Marks and co-workers (J.B.C. 253: 4214, 1978) published the only structure-activity study ever carried out for this series of compounds. It was confined mainly to amides, acids and amines and showed that relatively minor structural modifications (e.g., NMF to HMBA) could increase compound potency by a factor of 50 in the Friend erythroleukemia system. When it became apparent from our preclinical studies that attaining the sustained blood levels of HMBA that were required in vitro might be a problem in humans, we began a a further structure-activity study to see if unexplored structural modifications could produce an even more potent compound. Because considerable expertise already existed in the use of the HL-60 cell line for differentiation studies of this type (Dr. Breitman), this line was chosen as the in vitro test system.

It was soon discovered, contrary to what might be expected from the literature, that HMBA (used as a positive control in all experiments) killed ca. 90% of the test cells at concentrations which caused 99% differentiation of the remaining 10% viable cells. For this reason, a differentiation factor (DF) was devised which measures both differentiation and viability at a given concentration (DF = differentiation x viability). An ideal compound [producing 100% viability (1.0) and 100% differentiation (1.0)] would have a DF = 1.0. For HMBA (standard 4 day exposure), DF is maximum (0.40) at a concentration of 3 mM, giving 59% differentiation with 67% viability. At 5 mM, although 96% differentiation of

viable cells was achieved, 87% of the total cell population was dead, resulting in a DF value of only 0.12.

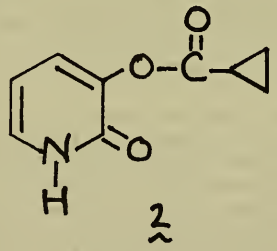
Seventeen HMBA analogs were synthesized and chemically characterized. In addition to bis-amides, these included bis-imides and hydrazides. Five of these compounds produced DF values in the HMBA range (0.3-0.5) but at concentrations approximating that of the parent (1-3 mM) so no significant increase in potency was achieved. The bis-hydantoin (1) was one of the more structurally interesting actives discovered. Work on this series has been suspended.



1

Pyridone Analogs. Early work by Marks showed that 2-pyridone would cause differentiation in Friend cells. More recent work by Sartorelli has shown that certain analogs of this compound are as potent as HMBA in the Friend cell line and appear to be just as active. Because of a long-standing interest in the antitumor properties of 2- and 4-pyridones, seven new compounds were synthesized and a total of 24 of these materials evaluated for differentiating properties in the HL-60 system. 1-Methyl-2-pyridone (MP), a compound shown by the Sartorelli group to produce 89% differentiation at 4 mM concentration, was used as a positive control in all experiments. In our studies, the DF value (see above) for MP remained approximately constant for 3, 4 and 5 mM concentrations (DF = 0.3) with percent differentiation increasing from 42% to 78% but with cell viability declining from 66% to 42%.

At least 12 of the pyridones tested from our series proved to be as good as MP from the point of maximum DF value and potency. Several compounds were significantly better than either MP or HMBA. The most interesting compound discovered thus far is the cyclopropyl analog, 2, with DF = 0.7 at 30 μM and 0.1 mM concentrations. Several 3-acyloxy-2-pyridones have proven active and this series will be explored further to find the optimum analog.



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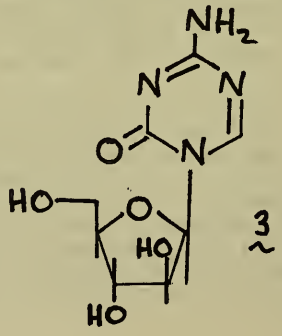


3

Ara-AC Activity Against Intracerebral Tumor Models (Drs. Driscoll, Plowman, Johns). Ara-AC is an LPET compound currently in preclinical toxicology. It has a hybrid structure (3) containing the sugar of ara-C and the heterocyclic base of 5-azacytidine (5-AC). Ara-AC was chosen as a clinical candidate based on DN2 level activity against all three of the human tumor xenografts in the NCI tumor panel. Because of known ara-C activity against intracerebral (IC) tumor models, ara-AC was evaluated in direct comparison with its two parent drugs against IC L1210 leukemia in mice (IC tumor implant; IP drug treatment). Although 5-AC was active (ILS = 70%) in this system, it was much less active than the other two compounds. Ara-AC and ara-C were most active on an "around-the-clock" treatment schedule. While each compound produced 60 day survivors, ara-AC did so over a greater dosage range (Table 1). This general superiority was also observed: a) on the qd 1-9 treatment schedule, b) when a greater tumor burden was used, and c) in the IC P388 model.

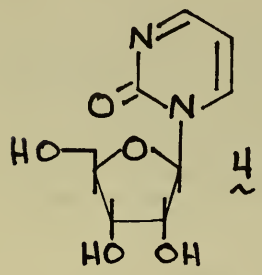
Table 1

Comparison of Ara-C and Ara-AC Activity Against IC L1210
Using the Q3HX8; D1,5,9 Treatment Schedule

Dose (mg/kg/injection)	Experiment 1 % ILS (LTS/6)*		Experiment 2 % ILS (LTS/6)*	
	Ara-C	Ara-AC	Ara-C	Ara-AC
75	2	0	Toxic	48
37.5	11	28	11	>640 (3)
18.75	>581 (5)	>581 (5)	>640 (5)	>640 (4)
9.38	218 (2)	>581 (5)	196	>640 (3)
4.69	115	263 (1)	146 (1)	251 (1)
2.34	70	193	107 (1)	>640 (3)
1.17	56	161 (1)	65	134
0.59	5	51	39	60
	[BCNU>581% (5)]		[BCNU>640% (4)]	

*10^4 cells IC implant. IP drug treatment. Long term survivors measured on day 60.

Zebularine and its Analogs (Drs. Driscoll, Marquez, Plowman, Haces, Johns). Zebularine (4) is a previously known compound which the LPET found had a number of interesting biological properties.



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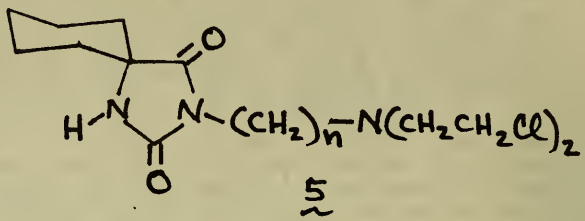


5

Although 4 has the simplest possible pyrimidine nucleoside structure, the compound possesses a wide spectrum of activities. Zebularine has: a) DN2 level L1210 and B16 melanoma activity, b) cytidine deaminase (Cdase) inhibitory activity and c) as good or better antiviral activity as ribavirin against both RNA and DNA viruses.

The Cdase activity of tetrahydrouridine (THU) is abolished in the presence of acid. Compound 4, as an acid stable compound, is being evaluated for oral administration with ara-C in an attempt to determine whether an oral ara-C formulation might be feasible. Initial in vivo experiments against L1210 leukemia indicate that zebularine is at least as efficacious as THU in potentiating oral ara-C activity. Syntheses of the ara- and 2'-deoxy analogs are in progress.

Potentiation of CNS Antitumor Activity (Drs. Driscoll and Cooney). AZQ and the nitrosoureas are drugs active against CNS tumors but whose limiting toxicities are peripheral. If a rescue agent could be devised which would destroy peripheral AZQ (or nitrosourea) but was not able to cross the blood-brain-barrier (BBB), it is possible that the CNS concentrations of these antitumor drugs might be raised relative to that achieved with currently administered doses. While this would shift the limiting toxicity to the CNS, this should be acceptable and even desirable for brain tumor patients. Our prototype rescue agent, MESNA, is an ionic sulfhydryl nucleophile which should not cross the BBB. Initial in vitro studies showed no effect of MESNA on AZQ toxicity so additional, more nucleophilic (but still ionic) agents are being sought.

Spiromustine Analogs (Drs. Haces, Driscoll). Spiromustine (5, n = 2) is an LPET compound undergoing both adult and pediatric Phase I brain tumor studies in the NIH Clinical Center.

This compound is very hydrolytically unstable and a limited synthetic analog study was undertaken to determine the effect of the number of hydantoin-mustard methylene spacer groups on: a) compound stability in human plasma and b) antitumor activity. Analogs of 5 with n = 2-5 were prepared. As n increased, the compounds became more unstable. Methylene number had essentially no effect P388 antitumor activity. The effect of methylene number on neurotoxicity (spiromustine limiting toxicity) will be evaluated as soon as an appropriate model is devised. The synthetic aspects of this project have been terminated.

Publication:

1. Sporn, M.B., Roberts, A.B. and Driscoll, J.S.: Growth factor and differentiation. In De Vita, V.T., Hellman, S. and Rosenberg, S.A. (Eds.): Cancer Principles and Practice of Oncology, Second Ed., Philadelphia, Lippincott, 1985, pp. 49-66.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03581-16 LPET

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James A. Kelley	Research Chemist	LPET, NCI
Others:	J. Driscoll	Section Head	LPET, NCI
	R. Heideman	I.P.A.	LPET, NCI
	J. Roth	Chemist	LPET, NCI

COOPERATING UNITS *(if any)*

Medicine Branch, Clinical Pharmacology Branch, Pediatric Branch, COP, DCT; Surgical Neurology Branch, NINCDS

LAB/BRANCH

Laboratory of Pharmacology and Experimental Therapeutics

SECTION

Medicinal Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

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 anti-tumor 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 pharmacokinetics, and (4) study reaction mechanisms of potentially useful sythetic transformations. Mass Spectrometry, gas chromatography and high-performance liquid chromatography, either alone or in combination, are emphasized techniques. Compounds of current interest are cytidine analogs, cytidine deaminase inhibitors, modified nucleosides, oligonucleotides, nitrogen mustards and differentiating agents. The kinetics and products of the acid-catalyzed isomerization of reduced pyrimidine ribosides and tetrahydrouridine have been determined.

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 chromatography (HPLC) and the combination of these techniques are the emphasized methods. Other analytical methods such as NMR, UV and IR spectroscopy are also employed.

Specific Objectives:

1. Analytical methods development for spiromustine and determination of human pharmacokinetics.

2. Central nervous system pharmacokinetics of hexamethylene bisacetamide in non-human primates.

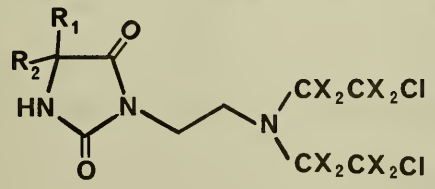
3. Analytical methods development and preclinical pharmacology for arabinosyl-5-azacytosine (Ara-AC).

4. Synthetic and collaborative project support.

Major Findings:

1. Analytical Methods Development for Spiromustine and Determination of Human Pharmacokinetics:

a. Analytical Methods Development (Drs. Kelley, Heideman): Spiromustine (spirohydantoin mustard, NSC 172112, 1a), a new candidate antitumor agent with apparent activity against central nervous system (CNS) tumors, is currently undergoing Phase I clinical trial. Because of the low dose that is administered to human patients and because of this compound's extreme plasma instability ($t_{1/2}$ = 6.5 min, pH 7.4, 37°), very sensitive and specific analytical methods are required to measure this drug in biological samples and define plasma elimination and CNS penetration. We have developed both a GC/MS method employing an isotopically labelled internal standard with selected ion monitoring, and a capillary GC method using a wide-bore fused silica capillary column with a nitrogen-phosphorus detector (NPD). Spiromustine-d_8 (1b) is used as an



1a $R_1, R_2 = -(CH_2)_5-$; X = H
1b $R_1, R_2 = -(CH_2)_5-$; X = D
1c R_1, R_2 = Et ; X = H

internal standard for the GC/MS procedure, while diethylhydantoin mustard (1c) is the internal standard for the GC-NPD method. The same extractive workup is carried out for both methods. Because of the plasma instability of spiromustine, biological samples must be rapidly extracted as they are obtained at the patient's beside. A single 2 min extraction with hexane-toluene (7:3, v/v) gives an average recovery of 86% for the 25-255 ng/ml range. Subsequent analysis by either analytical method allows reliable mesurement of 10 ng/ml levels of spiromustine in human plasma. Work is currently in progress to increase the sensitivity of the GC/MS method by substituting the more inert wide-bore capillary columns for the packed columns now used. A ten-fold increase in detection limits is anticipated.

b. Human Pharmacokinetics of Spiromustine in an NCI/NINCDS Phase I Clinical Trial (Drs. Kelley, Heideman, Curt, Collins and Kufta): Spiromustine has been administered to 22 patients with high grade primary CNS malignancy. It was given as an i.v. bolus q. 4 hr x 3 every 28 days at doses ranging from 3.0 - 15.6 mg/m^2. A fractionation of the dose has been employed in the hopes of minimizing the dose-limiting neurotoxicity reported by other clinical investigators. Dose fractionation places an added demand on the sensitivity required in the analysis of biological samples. Plasma samples from 8 different patients have been analyzed and the plasma concentration (C_p) versus time data from 5 of these were sufficient to define pharmacokinetic parameters (Table 1). A two-compartment open model where the $t_{1/2}$ for the terminal phase is only 12.5+ 3.3 min (n=5) adequately defines the plasma elimination curve. All measured plasma levels are in the nanogram per ml range with peak plasma levels exceeding 200 ng/ml (6 x 10^{-7}M) in some patients. Administration of spiromustine as a short (10-15 min) infusion is planned in order to validate this pharmacokinetic model since extrapolation to zero time appears to involve considerable uncertainty. An estimate of C_p at steady-state will allow determination whether the clearance of this rapidly eliminated drug has been overestimated.

Table 1. Pharmacokinetic Summary of Spiromustine Phase I Clinical Trial

Patient	Dose (mg/m^2)	Fractionated Dose (mg/m^2)	Peak[a] C_p (ng/ml)	$t_{1/2}(\alpha)$ (min)	$t_{1/2}(\beta)$ (min)	CL_{TB}[b] (ml/minXm^2)	V_{dss}[c] (l./m^2)
S.B	9.9	3.3	246	1.0	13.7	1096	11.7
J.S.	9.9	3.3	313	1.0	14.8	1679	12.8
J.A.	12.3	4.1	667	1.3	6.6	1201	6.0
D.H.	12.3	3.1[d]	168	0.6	13.5	2895	33.9
K.V.	12.3	4.1	766	0.8	13.9	1383	10.0

[a] measured
[b] total body clearance
[c] volume of distribution at steady-state
[d] administered 75% of normal dose

c. Plasma and CNS Pharmacokinetics of Spiromustine in a Pediatric Phase I Clinical Trial (Drs. Heideman, Kelley, Poplack): A pediatric Phase I clinical trial is also currently underway in which patients (< 19 yrs) are administered the drug as an i.v. bolus weekly x 3 on a 28 day cycle. Five patients have been treated at 4.5 or 5.5 mg/m^2 per i.v. bolus with either manageable or no apparent neurotoxicity. One patient treated at the higher dose has had plasma levels of spiromustine measured after two courses of drug on the same cycle. The resulting plasma elimination curves and the measured Cp's were similar to those observed on the adult Phase I study (see above), although mean total body clearance was somewhat higher (2740 versus 1650 ml/min x m^2). The plasma kinetics of additional patients will be examined to determine whether this difference is real. Also planned are studies to document spiromustine CNS penetration and elimination kinetics in those patients where an Ommaya reservoir or implanted shunt allows serial cerebrospinal fluid sampling.

2. Central Nervous System Pharmacokinetics of Hexamethylene Bisacetamide (HMBA) in Non-human Primates (Drs. Kelley, Poplack): HMBA, a synthetic polar-planar compound with potent differentiating activity in selected in vitro model systems, is the first compound of this type chosen by the NCI to undergo clinical trial. Analytical methods for measuring HMBA in plasma, urine and cerebrospinal fluid (CSF) have been further refined. The combination of solid-phase extraction (Sep-Pak) for isolation from the biological matrix, and wide-bore fused silica polar (OV-17) capillary column chromatography together with a nitrogen-phosphorus detector for GC analysis allows determination of less than 100 ng/ml HMBA in a 0.2 ml sample. Since the initial Phase I trials with of this agent indicate that metabolism may be more pronounced than that observed in preclinical studies, the developed analytical methods will be modified to concurrently measure partially and completely deacylated HMBA. This methodology will then be applied to a bioavailability and metabolism study of orally administered HMBA in the context of an NCI Phase I clinical trial.

A study to assess the ability of HMBA to penetrate the central nervous system in rhesus monkeys was completed. This study was prompted by the report that the major toxicity of HMBA in dogs was neurologic. Plasma and lumbar CSF kinetics were determined after both i.v. bolus and continuous infusion doses of HMBA in the same animals (Table 2). It can be seen that this compound rapidly and readily penetrates the CNS with a mean CSF:plasma ratio of 0.36 (n=6). Also interesting to note are that peak and steady-state CSF and plasma levels are inversely proportional to total body clearance, suggesting non-linear kinetics (or saturation of elimination mechanisms) in this species at these doses.

Table 2. CSF and Plasma Kinetics of HMBA in Rhesus Monkeys

IV "Bolus" (short infusion)

Monkey	Dose (mg/kg)	Cp(peak) (mcg/ml)	$t_{1/2}$ (min)	Cl_{TB} (ml/min/kg)	C_{CSF}(peak) (mcg/ml)	CSF:plasma ratio
1	226	357	59	6.5	53	0.24
2	240	745	103	3.8	124	0.55
3	485	1755	171	2.5	164	0.27

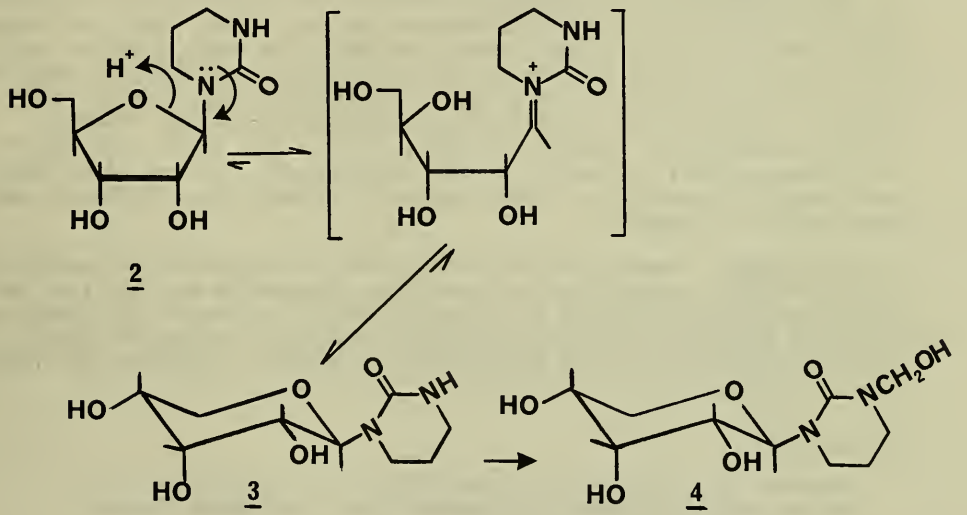
8 Hr Continuous Infusion

Monkey	Dose (mg/kg/min)	C_{ss} (mcg/ml)	Cl_{TB} (ml/min/kg)	C_{ss}(CSF) (mcg/ml)	CSF:plasma ratio
3	0.42	44	9.5	10	0.23
1	0.60	88	6.8	28	0.32
2	1.5	599	2.5	356	0.58

3. Analytical Methods Development and Preclinical Pharmacology for Arabinosyl-5-azacytosine (Ara-AC) (Drs. Kelley and Heideman): Ara-AC (NSC 281272) is a new synthetic nucleoside which combines the structural elements of two established antitumor agents, Ara-C (the arabinose sugar) and 5-AC (the triazine base). Chemically Ara-AC behaves like 5-AC, while it most closely resembles Ara-C in its antitumor and other biological properties. A sensitive and specific HPLC assay suitable for measurement of Ara-AC in clinical samples and for determination of human pharmacokinetics is currently under development. This assay utilizes reverse phase HPLC with UV detection at 240nm and 2'-deoxy-5-azacytidine, an analog with similar aqueous stability ($t_{1/2}$ (α) = 26 min versus 24 min for Ara-AC at pH 7.4, PBS, 37°), as an internal standard. A major effort is being devoted to sample cleanup since removal of interfering endogenous materials will be the major determinant of sensitivity. On-line solid-phase extraction with column switching is anticipated as a solution to this problem. The developed analytical method will then be validated and applied to a preclinical pharmacology study in a rhesus monkey model to determine the extent of Ara-AC CNS penetration. This will be of considerabl interest because of this compound's high activity in the murine intracerebral L1210 leukemia model.

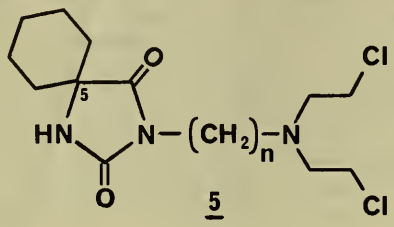
4. Synthetic and Collaborative Project Support

a. Furanose - Pyranose Isomerization (Drs. Kelley, Marquez, Driscoll): Unsaturated pyrimidine, diazepinone and cyclic urea ribosides undergo an acid-catalyzed isomerization from a β-ribofuranose to a predominantly β-ribopyranose form. Cytidine deaminase inhibitors which belong to the above classes of compounds can be almost completely deactivated by this isomerization. The pH dependent isomerization kinetics were investigated using 2 as a model compound. The resulting decomposition products were isolated by semi-preparative HPLC and chemically characterized. At pH 1 (37°), ribofuranoside 2 underwent rapid isomerization ($t_{1/2}$ = 10 min) to the β-pyranose 3, which was slowly reductively formylated by the formaldehyde preservative in the buffer. It was also demonstrated that 3 eventually achieves equilibrium with 2. This acid-catalyzed equilibrium was also investigated for tetrahydrouridine (THU) under conditions approximating those seen after oral administration. THU underwent isomerization with biphasic kinetics ($t_{1/2}$ (α) = 30 min) to two major products in simulated gastric fluid (pH 1.2, 37°). These THU isomers are still in the process of being characterized.



b. Spirohydatoin Mustard Analogs (Drs. Kelley, Heideman, Haces, Driscoll): The human plasma stability and the electron impact mass spectral characteristics of a series of spirohydantoin mustard analogs (5) have been determined and related to structure. The in vitro plasma half-lives of analogs n=2-5 were measured at 37° at clinically achieveable concentrations of 1mcg/ml using the capillary GC-NPD analysis developed for spiromustine (see above). Spiromustine itself was the most stable homolog ($t_{1/2}$ = 6.5 min). As the methylene unit spacer increased, the stability of these analogs approached that of nitrogen

mustard ($t_{1/2}$ = 1.6 min). Substitution at the 5-position of the hydantoin ring had little effect on mustard stability for n=2. A unique bicyclic ion (6) was observed in the mass spectra of all of these analogs. This fragment ion predominated for n=3, but its abundance could only be correlated with ring size and not mustard stability.



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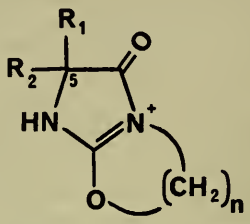

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c. Applications of Fast Atom Bombardment (FAB) Mass Spectrometry to Nucleoside and Nucleotide Analysis (Dr. Kelley): The MCS continues to have an extensive program in the synthesis of new nucleosides and nucleotides. Rapid and simple methods employing FAB mass spectrometry continue to be investigated and utilized for the characterization of underivatized nucleic acid constituents. Negative ion FAB mass spectrometry has been applied to determine the structure and purity of chemically and enzymatically synthesized NAD analogs. Chemical methods to enhance the sequence-indicating fragment ions in FAB mass spectra of oligoribonucleotides are currently under investigation. The use of FAB mass spectrometry to quantitate selected compounds isolated from a biological matrix also remains an interest.

d. 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 S-adenosyl methionine derivatives, inositol phosphates, neplanocin analogs, 4-pyridone derivatives, sugar phosphonates and photo-affinity labelled vinblastine derivatives.

Publications:

1. Huguenin, P.N., Jayaram, H.N. and Kelley, J.A.: Reverse phase HPLC determination of 5,6-dihydro-5-azacytidine in biological fluids. J. Liquid Chromatogr. 7:1433-1453, 1984.

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4. Litterst, C.L., Roth, J.S. and Kelley, J.A.: Distribution, elimination, metabolism and bioavailability of hexamethylenebisacetamide in rats. Inv. New Drugs, in press.

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7. Kelley, J.A., Roth, J.S. and Litterst, C.L: Gas chromatographic determination of hexamethylene bisacetamide in plasma and urine. Analyt. Lett., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07167-01 LPET

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Enzyme Inhibitors as Potential Anticancer Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

PI:	V. E. Marquez	Visiting Scientist	LPET, NCI
Others:	J. S. Driscoll	Head, Medicinal Chemistry Section	LPET, NCI
	C. H. Kim	Visiting Fellow	LPET, NCI
	C. K. H. Tseng	Visiting Fellow	LPET, NCI
	R. Fuller	Chemist	LPET, NCI
	S. Petraglia	Chemist	LPET, NCI

COOPERATING UNITS *(if any)*

Laboratory of Biological Chemistry

LAB/BRANCH

Laboratory of Pharmacology and Experimental Therapeutics

SECTION

Medicinal Chemistry Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.2	3.2	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 objective of this project is to design and synthesize mechanism-based inhibitors of enzymatic reactions that are critical for the growth of neoplastic cells. The resulting compounds are intended to be used as therapeutic agents and research probes. The following topics are of current interest: 1) Synthesis of cyclopentenyl nucleosides (Neplanocin A analogs), 2) Dinucleotide analogs of NAD as IMPD inhibitors, 3) Transition-state inhibitors of cytidine triphosphate (CTP) synthetase, 4) Synthesis of diazepinone nucleosides as fradulent uridine and cytidine analogs, and 5) Synthesis of stable analogs of the 2',5'-oligo-adenylate trimer as inducers of interferon production.

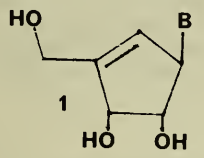
PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

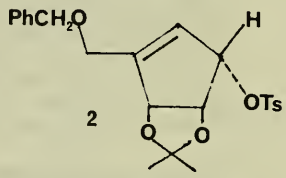
The objective of this project is the exploitation of recent biochemical findings concerning enzymatic mechanisms that are enhanced in rapidly proliferating cells, in order to design appropriate inhibitors for key enzymes. A suitably altered substrate (mechanism-based inhibitor) is selected as a candidate target and synthesized. The selected candidates are designed as reversible transition-state inhibitors or irreversible (K_{cat}) suicide inhibitors. A correlation between structure and activity in the series of inhibitors prepared serves to delineate the course of future synthetic work.

Major Findings:

Synthesis of Cyclopentenyl Nucleosides (Neplanocin A Analogs) (Drs. Tseng and Marquez). The original synthetic schemes for neplanocin A (1a) and the cyclopentenyl (CPE-) pyrimidines 1b and 1c were shortened considerably by the use of a direct displacement approach that employed the cyclopentenyl α-tosylate 2 and the alkali metal salts of the corresponding purine and pyrimidine bases.



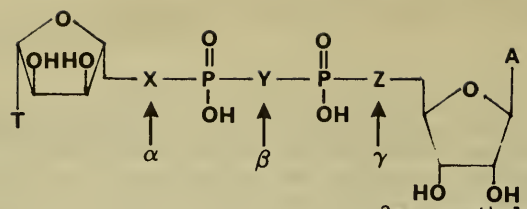
1a, B = adenine (neplanocin A)
1b, B = uracil (CPE-Uracil)
1c, B = cytosine (CPE-cytosine)
1d, B = thymine
1e, B = 5-FU
1f, B = 5-IU
1g, B = 3-deazaadenine



This new approach is currently being extended to the syntheses of other pyrimidines (e.g., 1d - 1f) and to the important target, 3-deazaneplanocin (1g). Conversion of the cyclopentenyl riboside isosteres into the arabinosyl and 2'-deoxy analogs will follow.

Biological studies (Drs. J. Moyer and R. Cysyk, LBC) with CPE-uracil (1b) and CPE-cytosine (1c) indicated that both compounds behaved as inhibitors of the enzyme uridine kinase. Although the CPE-uracil was the less potent of the two, it was attractive for its almost total lack of cytotoxicity. A marked decrease in labeled uridine uptake from intestine, liver, and spleen was observed in vivo following the administration of CPE-uracil at doses ranging from 100 to 300 mg/kg. In vitro studies conducted by Dr. Glazer (LBC) and confirmed in vivo by Dr. Moyer demonstrated that CPE-cytosine is a very potent inhibitor of CTP synthesis whose mechanism appears to be consistent with the irreversible inactivation of the enzyme. CPE-cytosine is a powerful differentiator of HL-60 cells in vitro (Dr. Glazer, LBC) (10^{-7}M). Additionally, following a qd 1-5 treatment schedule at a dose of 1 mg/kg in mice bearing L1210 leukemia, an ILS of 67% was observed (Dr. Moyer, LBC). These and other biological results have provided incentive to continue the syntheses of other purine and pyrimidine analogs bearing the novel cyclopentenyl ring.

Dinucleotide Analogs of NAD (Drs. Tseng and Marquez). The preparation of all possible phosphonate analogs of the active metabolite of tiazofurin, thiazole-4-carboxamide adenine dinucleotide (TAD), was completed with the synthesis of β-methylene TAD (3b).

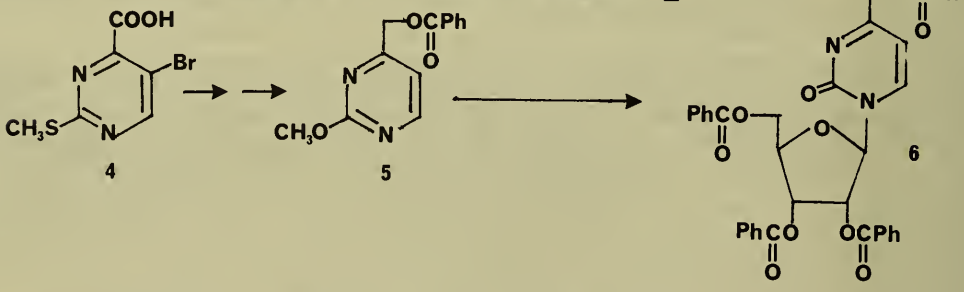


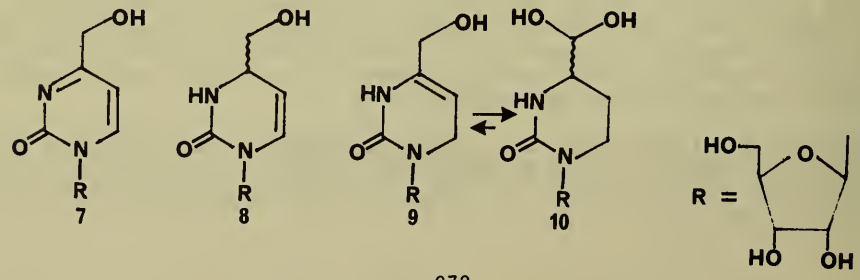
A = adenine
T = thiazole-4-carboxamide

3a, α-methylene TAD
b, β-methylene TAD
c, γ-methylene TAD

Synthesis of β-methylene TAD was accomplished by the coupling of adenosine 5'-(α,β-methylene)diphosphate with protected tiazofurin (2',3'-acetonide) in the presence of dicyclohexylcarbodiimide (DCC). All the analogs (3a-c) retained their inosine monophosphate dehydrogenase (IMPD) activity, plus they were more resistant than TAD towards enzymatic cleavage. In particular, the β methylene TAD (3b), was equipotent to TAD as an IMPD inhibitor and was totally resistant to phosphodiesteric cleavage by both venom phosphodiesterase and TAD-phosphodiesterase (Dr. Cooney, LPET). Since it is believed that TAD breakdown is responsible in part for either the lack of response or the development of resistance to tiazofurin, this compound offers a unique opportunity to test this hypothesis. Additionally, 3b was cytotoxic to P388 cells in vitro (IC_{50} = 45 μM) which meant that despite being a charged molecule it was transported across the cell membrane. Efforts are now directed towards preparing a more lipid-soluble form of this compound with the intent of improving its penetrability into the cell.

Transition-State Inhibitors of Cytidine Triphosphate Synthetase (Drs. Kim and Marquez). The key intermediate for this project, (6), was obtained by a Hilbert-Johnson reaction employing a protected halogenosugar and the heterocycle 5 which was prepared in four steps from compound 4.

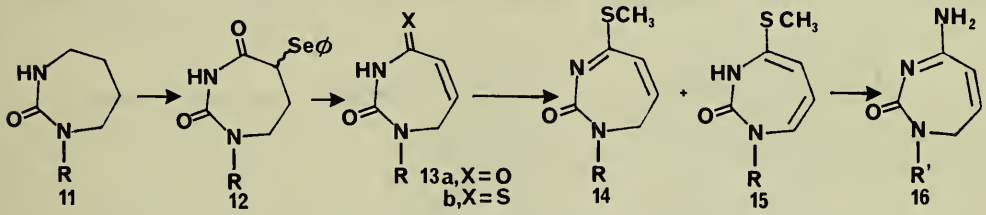




The deblocking of 6 produced compound 7, whereas compounds 8 and 10 were obtained from 6 in a three step sequence which involved a partial reduction with sodium borohydride, separation of isomers, and deblocking. The 4-hydroxymethyl substituent in compounds 7 and 9 is really a masked aldehyde group that can be generated through an intramolecular redox reaction. In 7, however, the stability of the aromatic ring prevents aldehyde formation whereas in the partially reduced compound, 9, the aldehyde is readily unmasked and is immediately hydrated to give the hemiacetal 10.

Compounds 7, 8 and 10 have been submitted for testing (Dr. Cooney) to see whether CTP pools will be affected or not. Transformation of the pyrimidine hydroxyl group in 7 and 8 into halogens, phosphonate groups, etc., is planned for the near future.

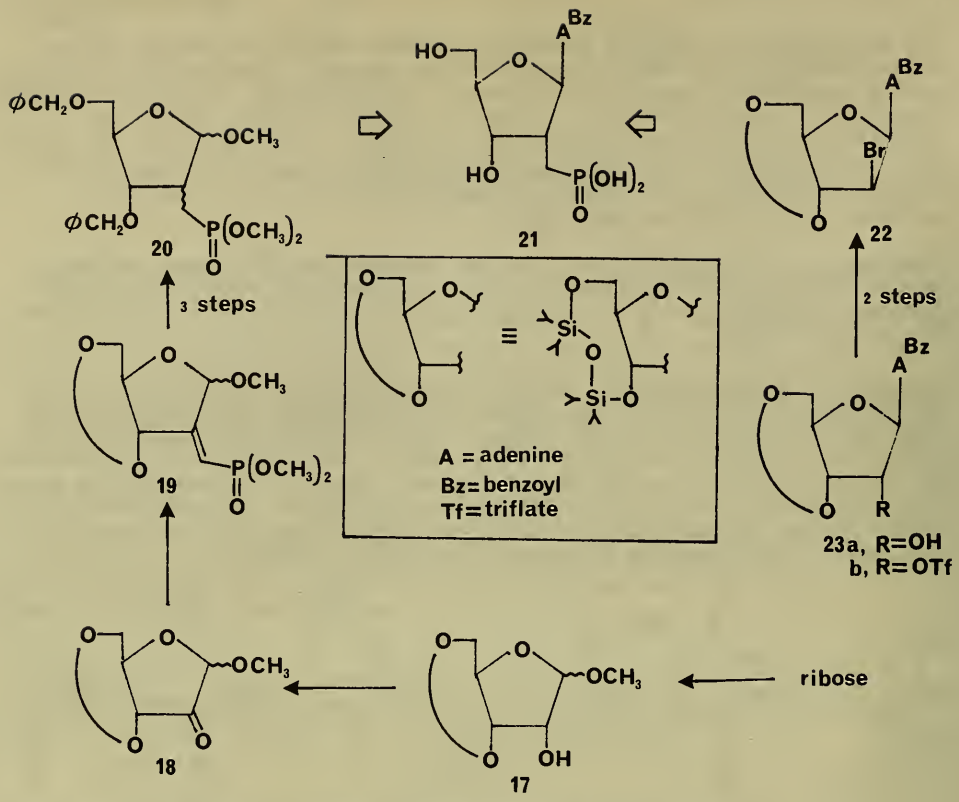
Synthesis of Diazepinone Nucleosides as Potential Antitumor Agents (Drs. Kim and Marquez). The synthesis of ring-expanded cytidine (16) was accomplished from the nucleoside 11 in seven steps. After oxidation with phenylseleninic anhydride, compound 12 was obtained; following a two-step oxidation-elimination of the phenyl selenium substituent as PheSeOH, the ring-expanded uridine analog 13a was generated. Thiation of the 13a and treatment of this compound with methyl iodide, produced a mixture of 14 and 15, which was readily aminated and deblocked in one operation to give 16, the desired compound.



R = 2',3',5'-tri-O-benzoyl-β-D-ribofuranosyl
R' = β-D-ribofuranosyl

Ring-expanded cytidine was a poor inhibitor of uridine-cytidine kinase (Dr. J. Moyer) and showed poor growth inhibitory properties against L1210 cells in vitro (37% inhibition at 1 mM). This compound will be evaluated as substrate or inhibitor of cytidine deaminase. It is expected that this project will terminate soon.

Synthesis of Phosphonate Analogs of the 2',5'-Oligoadenylate Trimer Core (Drs. Tseng and Marquez). Progress towards the target $(2'\text{-}5')A_3$ analog in which the phosphate ester linkages between the 2'-hydroxyl group and the adjacent 5'-phosphate of the trimer are replaced by hydrolytically stable methylene linkages, has centered around the synthesis of the monomer 21. Two independent approaches, which led to the syntheses of precursors 20 and 22, were initiated. Compund 22 offers the attractive feature that the resulting α-stereochemistry of the phosphonate group in 21 will be uniquely established. However, several attempts to displace the bromine atom of 22 by lithium dimethyl methylphosphonate, or even by the smaller cyanide anion, have failed.



In the second approach, compound 20 has been prepared from D-(-)-ribose in seven steps. Although in this approach the selective stereochemical control is lost, the chemistry is more favorable and efforts to condense adenine with the chlorosugar derived from 20 are being pursued. In view of the lack of controlled stereochemistry characteristic of this route, separation and identification of isomers is anticipated. Relative to the naturally occurring material, the target compound is expected to yield an enzymatically more stable trimer core capable of inducing production of interferon in treated cells.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07102-10 LPET

PERIOD COVERED: October 1, 1984 - September 30, 1985

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

Tubulin Structure and Function as Sites for Pharmacologic Attack

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Hamel	Senior Investigator	LPET, NCI
Others:	J. K. Batra	Visiting Fellow	LPET, NCI
	C. Duanmu	Visiting Fellow	LPET, NCI
	C. M. Lin	Biologist	LPET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Pharmacology and Experimental Therapeutics

SECTION: Biochemical Pharmacology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, MD 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	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 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, its two tightly bound guanine nucleotides, and drugs which inhibit mitosis. Substitution of ATP and ATP analogs for GTP in glycerol-induced polymerization was demonstrated. This will permit structure-function studies of nucleotide interactions at the exchangeable site with more readily available ATP analogs. Degradation of the nonexchangeable GTP of tubulin by phosphofructokinase was observed, representing the first approach to this nucleotide which does not require the denaturation of the protein. Studies on the interactions of a large group of antimitotic agents with tubulin continued. The classes of compounds examined were combretastatin congeners; derivatives of 6-benzyl-1,3-benzodioxole and related compounds; derivatives of 5,6-diarylpyridazin-3-one; 2,4-dichlorobenzylthiocyanate; and alkyl carbamates of aromatic amines. A microtubule-associated protein which causes the formation of microtubule bundles was purified to homogeneity. Microtubule-associated protein(s) with nucleoside monophosphate kinase activity were resolved into components with different substrate specificity, and one component was purified to homogeneity. Maytansine was found to inhibit entry and exit of GDP and GTP at the exchangeable nucleotide binding site. Efforts to separate the α and β subunits of tubulin continued, and efforts to prepare GTP analogs derivatized at the γ-phosphate were initiated.

GPO 614-918

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). The remainder of the GTP (the nonexchangeable nucleotide) is not altered during tubulin polymerization and can only be removed from tubulin by destroying the protein. Its function is unknown.

We have confirmed that in glycerol-induced tubulin polymerization (a reaction condition in which MAPs are not required) high concentrations of ATP can directly replace GTP in supporting tubulin polymerization. Contrary to interpretations in the literature, we have found that this polymerization reaction requires an interaction of ATP at the exchangeable nucleotide site and probably involves hydrolysis of ATP to ADP. In ATP-supported polymerization, GDP in the exchangeable site is displaced by ATP; and GDP is a potent inhibitor of the reaction. In addition, we have found that ribose- and polyphosphate-modified ATP analogs closely mimic comparable GTP analogs in their effects on tubulin polymerization. This implies that other ATP analogs may also be used to define in further detail structural requirements for the nucleotide at the exchangeable site. This is of importance since ATP analogs are much more common than GTP analogs and should save a considerable amount of synthetic work.

We have observed that when phosphofructokinase and fructose-6-phosphate are added to microtubules, even when GTP in the reaction mixture has been exhausted, there is an immediate rapid and extensive depolymerization of the microtubules. Since both the sugar and enzyme are required for this to occur, nucleotide breakdown is implied. This in turn suggests that it is the nonexchangeable GTP which may be involved in the disassembly process catalyzed by phosphofructokinase, and we are attempting to document whether nonexchangealbe site GTP breakdown is occurring.

New antimitotic agents continue to be an active area of interest in the laboratory. We are currently studying the following classes of drugs:

1) Analogs of combretastatin. Combretastatin (NSC 348103) is a natural product isolated by G. R. Pettit of Arizona State University from the South African tree Combretum caffrum; and we have demonstrated that combretastatin is a potent inhibitor of tubulin polymerization. Dr. Pettit's group has now purified, but not yet fully characterized, a number of additional compounds from Combretum caffrum. We have established that at least two of these as yet unidentified agents are considerably more potent than combretastatin itself as tubulin inhibitors.

2) A large number of derivatives of 6-benzyl-1,3-benzodioxole have been prepared by Dr. L. Jurd of the Department of Agriculture as potential insect sterilants. A significant number of these compounds have antineoplastic activity and inhibit tubulin polymerization. They are most analogous to podophyllotoxin structurally, and like podophyllotoxin, inhibit both tubulin-dependent

GTP hydrolysis and the binding of colchicine to tubulin. Initial studies established minimal structural requirements for the simplest benzylbenzodioxole derivatives (a 1-3 carbon substituent at position 5, and a methoxy group at position 4' in the benzene ring) which have either an unsubstituted one carbon bridge between the benzene and benzodioxole rings or one or two methyl groups at this bridge position. Additional methoxy substituents on the benzene ring at the 3' and 5' positions, which seemingly increase the structural analogy to podophyllotoxin, resulted in almost complete loss of activity.

Dr. Jurd has more recently prepared a large series of derivatives with a morpholino group at the bridge carbon and no substituent at the 5 position. The variation is in the substituents on the benzene ring. The most active compounds in this group are also potent tubulin inhibitors, and they too seem most analogous to podophyllotoxin in their interactions with the protein. These compounds permit a more detailed structure-function analysis than is possible with the much more complicated natural product podophyllotoxin, and provide promising leads for further synthetic work with this group of drugs. The morpholino compounds themselves, however, have limited cytotoxicity and do not appear to cause mitotic arrest in cultured cells.

Dr. Jurd has also prepared a group of compounds with a third ring (of variable structure) fused to the benzodioxole moiety. The benzene ring is attached directly to this third ring. Several of these agents have potent antitubulin activity, and most of the compounds active against tubulin in vitro also cause mitotic arrest. All active compounds have three methoxy groups, attached at positions 3', 4' and 5', on the benzene ring. Although both the third fused ring and the trimethoxy structure appear to substantially increase their analogy to podophyllotoxin, these new agents are more comparable to colchicine in their effects on tubulin-dependent GTP hydrolysis; for, like colchicine, they stimulate rather than inhibit this reaction even while inhibiting the microtubule assembly reaction normally coupled to GTP hydrolysis.

3) Dr. L. Powers of SDS Biotech Corporation has prepared over 100 derivatives of 5,6-diarylpyridazin-3-one as potential antihypertensive agents. Some of these compounds were found to be potent herbicides, and this seemed to be a consequence of inhibition of mitosis in plant tissues. Several members of this class were then submitted to the NCI for screening, and some of these were found to have antineoplastic activity. We have found that a number of these drugs inhibit mitosis of mammalian cells in culture and the polymerization of tubulin in vitro. They potently stimulate tubulin-dependent GTP hydrolysis; but they probably bind at a previously undescribed site on tubulin for they do not inhibit the binding of either colchicine, vinblastine or maytansine to the protein. Most active compounds possess a nitrile group at position 4; and in vitro interactions with tubulin are significantly enhanced by chloride substituents on the phenyl rings. There is little overlap between compounds most active against mammalian tubulin and those which are most active in inhibiting mitosis in plant cells.

4) The compound 2,4-dichlorobenzylthiocyanate (NSC 145813) has been found to inhibit mitosis in murine leukemia cells and, in collaboration with other investigators, to cause clumping or bundling of microtubules in CHO cells.

In addition, certain lines of CHO cells with mutants in tubulin genes are resistant to NSC 145813. The effects of this agent on in vitro tubulin polymerization are highly unusual. There is minimal change in the reaction unless drug and tubulin are preincubated (in the absence of GTP to avoid polymerization). With a preincubation at 37° lasting at least one hour, however, low concentrations of the drug completely inhibit tubulin polymerization. This inhibition can be prevented with dithiothreitol. We believe that NSC 145813 is acting as a highly specific alkylating agent, attacking one or more of the sulfhydral groups of tubulin, and that this reaction produces its antimitotic and antitubulin effects. We are in the process of obtaining radiolabeled drug to use to prove this hypothesis. We will also attempt to document which one of the subunits of tubulin acts as the target of the drug.

5) A number of compounds with very different structures have been found to have antineoplastic and antimitotic properties and to inhibit tubulin polymerization. Their only common feature is that they are alkyl carbamates of aromatic amines. We have obtained a computer search of the NCI drug collection, and there are at least 140 compounds with structural features which suggest possible antimitotic activity. We are currently screening these compounds in an effort to identify additional drugs with antitubulin properties.

Another area of active interest in the laboratory is microtubule-associated proteins. We are currently purifying two components from this large group of proteins. The first of these causes the formation of microtubule bundles (distinc microtubules which aggregate laterally). The active component does not bind to DEAE-cellulose, does adhere to hydroxyapatite, is heat-stable, and can be reconstituted after denaturation in 8 M urea. Most recently the protein has been further purified on a sizing column by high pressure liquid chromatography. This last step appears to result in an electrophoretically homogeneous protein consisting of two subunits of molecular weights of approximately 20,000 and 30,000. This last step will now be attempted on a preparative scale.

The second MAP of interest to us is the enzyme nucleoside monophosphate kinase (NMK). Our interest in this enzyme began when we observed that extensive nucleotide interconversions occurred in microtubule protein preparations if both radiolabeled GTP and adenosine 5'-[β,γ-imido]triphosphate were included in the reaction mixture. Similar results were obtained when AMP was substituted for the ATP analog, probably indicating the latter's contamination with AMP. (Repurification of the analog resulted in the disappearance of the nucleotide interconversions observed with the impure compound.) NMK does not bind to DEAE-cellulose or hydroxyapatite. When NMK is applied to cibacron blue-agarose, active components which are purified to electrophoretic homogeneity are obtained. The initial protein preparation accepts as substrates AMP, ADP, ATP, GMP, GDP, and GTP, with A-G mixtures preferred to AMP-ATP or GMP-GTP. Following cibacron blue-agarose chromatography substrate specificity changes, suggesting the resolution of the NMK into two or more different components.

Additional areas of activity in the laboratory.

1) The antimitotic compound maytansine was found to inhibit completely the

entry or exit of GDP or GTP at the exchangeable nucleotide binding site of tubulin. 2) Initial, unsuccessful attempts were made to prepare GTP analogs derivatized at the γ-phosphate with hydrophobic groups. Previous studies from this laboratory have indicated that such compounds might be potent inhibitors of polymerization through interactions at the exchangeable site. 3) We have observed that under certain reaction conditions tubulin with GDP in the exchangeable site will co-polymerize with tubulin bearing GTP. We are attempting to define these conditions in detail. 4) 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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2. Hamel, E., Lustbader, J. and Lin, C.M.: Deoxyguanosine nucleotide analogs: potent stimulators of microtubule nucleation with reduced affinity for the exchangeable nucleotide binding site of tubulin. Biochemistry 23: 5314-5325, 1984.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07104-10 LPET

PERIOD COVERED

October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D. T. Vistica	Pharmacologist	LPET, NCI
Others:	S. Ahmad	Visiting Fellow	LPET, NCI
	H. Schalch	Biologist	LPET, NCI
	J. Hill	Biologist	LPET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology and Experimental Therapeutics

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.0	1.4

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.)*

Metastatic migration of the murine L1210 leukemia and an L-phenylalanine mustard resistant variant (L1210/L-PAM) from the peritoneal cavity and infiltration into the liver results in a 2-fold increase in the glutathione (GSH) content of both tumor cells. These increases in the cellular content of GSH are accompanied by a 2-fold increase in the resistance of these tumor cells to L-phenylalanine mustard. Cell surface labeling studies indicate that cells isolated from the liver have a 5-fold greater content of surface sulfhydryl groups as compared to their ascitic counterparts. The former can be sensitized to L-phenylalanine mustard by blocking these sulfhydryl groups with 6-6' dithiodinicotinic acid.

These results demonstrate a determinant role of the hepatic microenvironment in the maintenance of tumor cell GSH, cell surface sulfhydryl groups and the sensitivity of these tumor cells to L-phenylalanine mustard.

PHS 6040 (Rev. 1/84)

GPO 914-918

Objectives:

This project is designed to examine the role of the environment of the host organ on tumor cell proliferation and sensitivity to chemotherapeutic regimens.

Methods Employed:

A. Maintenance of Tumors

The murine L1210 leukemia and a line developed for resistance to L-phenylalanine mustard (L1210/L-PAM) were maintained in male CDF_1 mice by weekly intraperitoneal injection of 1×10^5 and 1×10^6 cells respectively.

B. Isolation of Tumor Cells from the Ascites and Liver

Animals bearing 7 day tumors were anesthesized with 5% chloral hydrate. Tumor cells were removed from the peritoneal cavity and used for determination of cellular glutathione and sensitivity to L-phenylalanine mustard as described below.

Tumor cells were isolated from the liver following sequential perfusion calcium and magnesium free Hank's balanced salt solution containing 1mm EGTA followed by 100 units/ml collagenase in William's Medium E. The liver was removed and teased apart in Dulbecco's phosphate buffered saline (without calcium and magnesium) containing 0.1mm sulfhydryl modified bovine serum albumin and 10mm glucose. The cell mixture, containing tumor cells and hepatocytes, was centrifuged at 300 x g and the cells washed twice in Dulbecco's phosphate buffered saline containing 0.1mm sulfhydryl modified bovine serum albumin and 10mm glucose. The cells were then resuspended in 50ml of the same buffer system in a 50ml Falcon conical centrifuge tube and allowed to stand undisturbed at 25°C for 60 minutes. The top 30ml containing tumor cells was removed and utilized for determination cellular glutathione and sensitivity to L-phenylalanine mustard as described below.

C. Determination of Cellular Glutathione Content

Tumor cells from the ascites and liver ($2x10^7$) were washed twice in Dulbecco's phosphate buffered saline containing 0.1mm bovine serum albumin and red blood cells removed by hypertonic lysis in 0.87% ammonium chloride for 5 minutes at room temperature. Cells were then washed once in Dulbecco's Phosphate buffered saline, pelleted by centrifugation at 300 x g for 10 minutes and lysed in distilled water. Sulfosalicylic acid was added to a final concentration of 3% and protein removed by centrifugation at 12,000 x g for 2 min. Glutathione (GSH) and its disulfide (GSSG) were assayed by the standard spectrophotometric assay of Griffith.

D. Evaluation of L-phenylalanine Mustard Cytotoxicity

Cells, isolated from the ascites and liver, as described above, were suspended in RPMI 1630 medium containing 16% heat-inactivated fetal calf serum, 40 µg/ml gentamicin and 50 µM B-meraptoethanol. Cells were exposed to L-phenylalanine

mustard for 2 days and surviving cells estimated by clonal growth in soft-nutrient agar for 2 weeks.

E. Determination of Membrane Sulfhydryl Content of Cells Isolated from the Ascites and Liver

Cells, in Dulbecco's phosphate buffered saline containing 0.1mm sulfhydryl modified bovine serum albumin were exposed to 100 μm [^{14}C] 6,6'-dithiodinicotinic acid for 15 minutes at 37°C. Cells were washed twice in the same buffer system and treated with 1mm glutathione to release the bound radiolabel. Cells were then removed by centrifugation thru versilube F-50 silicone oil and the supernatant was counted by liquid scintillation spectrometry.

Major Findings:

1. Metastatic migration from the peritoneal cavity and infiltration into the liver results in a 2-fold increase in the GSH content of both L1210 and L1210/L-PAM tumor cells.

2. These increases in the cellular content of GSH are accompanied by a 2-fold increase in the resistance of these tumor cells to L-phenylalanine mustard.

3. These observed differences in GSH content and sensitivity to L-phenylalanine mustard in cells isolated from the ascites and liver are observed only in vivo since short term culture results in cells with similar GSH content and sensitivity to L-phenylalanine mustard.

4. Cell surface labeling studies indicate that cells isolated from the liver have a 5-fold greater content of surface sulfhydryl groups as compared to their ascitic counterparts. The former can be sensitized to L-phenylalanine mustard by blocking these sulfhydryl groups with 6-6' dithiodinicotinic acid.

5. These results demonstrate a determinant role of the hepatic microenvironment in the maintenance of tumor cell GSH, cell surface sulfhydryl groups and the sensitivity of these tumor cells to L-phenylalanine mustard.

Publications:

1. Somfai-Relle, S., Suzukake, K., Vistica, B.P., and Vistica, D.T.: Glutathione-conferred resistance to antineoplastics: Approaches toward its reduction. Cancer Treat. Rev. 11: 43-54, 1984.

2. Green, J.A., Vistica, D.T., Young, R.C., Hamilton, T.C., Rogan, A.M., and Ozols, R.F.: Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion. Cancer Res. 44: 5427-31, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07119-06 LPET

PERIOD COVERED: October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	C. L. Litterst	Pharmacologist	LPET, NCI
Others:	J. Uozumi	Visting Fellow	LPET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Pharmacology and Experimental Therapeutics

SECTION: Biochemical Pharmacology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

Although the toxic effects of cisplatinum on 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 conditions and after various pretreatments 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 sex and tissue specificity of platinum binding to DNA, the effect of cisplatinum on renal ATPase function in vivo and in vitro and the blockage of cisplatin-induced renal toxicity by sodium thiosulfate.

Studies on the mechanism of action of cisplatin:

Much work has been done to document the DNA binding of cisPt and to establish this as the probable mechanism of the antitumor effect. Similarly, much effort has been invested in documenting the various toxicities produced by cisPt, although the mechanisms of these effects have not yet been satisfactorily established. There is an obvious interest in attempting to establish whether there is any relation between DNA binding and renal toxicity. We have therefore investigated the relation between DNA binding as measured by an ELISA technique which is specific for the N-6 DNA adduct with cisPt, and various parameters of platinum toxicity and pharmacokinetics. We have documented an apparent sex-related specificity in the uptake of cisPt into renal nuclei between male and female rats. Although the total renal concentration of Pt is greater in female rats, the DNA binding in females is less than in males. This finding is consistent with clinical response of ovarian and testicular cancer patients and with the differential response observed between older (post-menopausal) and younger (pre-menopausal) ovarian cancer patients. The implication is that there is a hormonal influence on Pt binding or transport which is responsible for the lesser amount of binding in females. A related organ-specificity also was noted, with testicles accumulating substantially greater amounts of DNA adducts that did ovaries. This is again consistent with the greater clinical response seen in testicular cancer patients than in ovarian patients. Finally, in both kideys and gonads of males and females, DNA binding but not whole tissue platinum content, appears to be dose dependent, with a dramatically elevated increase in binding at high cisplatin doses. This suggests, among other things, that the transport of platinum into the nucleus, or the cytosolic binding of platinum, may be saturable. These early results are currently in manuscript form, and experiments are continuing to investigate the mechanism behind these findings.

The effect of cisplatin on content and function of renal ATPase was investigated as a possible explanation for the renal toxicity of the drug. When purified ATPase or when ATPase from tissue homogenates were incubated in vitro with cisplatin decreases in ATPase activity were observed. However, either very high concentrations of cisplatin (400-600 uM) or very long times of incubation (2-3 hrs.) were required before any changes were noted. In vivo, cisplatin produced a decrease in ATPase that occurred several days after drug administration and 1-2 days after significant elevations of BUN occurred. Renal platinum concentrations were below the concentrations required to produce significant decreases in activity when tested in vitro. It was concluded that cisplatin probably does not interact with ATPase as a primary site of toxicity, but that cisplatin does have the ability to decrease the activity of this enzyme as a secondary effect.

Sodium thiosulfate (STS) was shown to completely block the cisplatin induced rise in BUN and to prevent cisplatin-induced deaths. The mechanism of this interaction was investigated by examining the subcellular distribution of platinum in rats receiving cisplatin and in rats receiving cisplatin plus STS. Renal platinum concentrations in STS pretreated rats were less in nuclei, mitochondria, and microsomes during the first 8 hours after cisplatin administration. Similar decreases in platinum content were not consistently observed in liver. The differences in distribution were statistically signif-

icant but were of a relatively small magnitude, so the biological significance is questionable. Because of this small changes in distribution, it is probably not realistic to ascribe the dramatic blockage of renal toxicity entirely to changes in subcellular distribution of platinum.

Pharmacokinetics and toxicity of experimental drugs:

Tetraplatin is a new analog of cisplatin that has shown activity in animal tumor systems which are resistent to cisplatin. We have conducted extensive preclinical toxicity studies of this compound in comparison with cisplatin and CHIP, another cisplatin analog with chemical similarity to tetraplatin. We evaluated the effect of 4 equimolar doses of the drugs on renal histology, urinary and plasma enzymes, and renal slice function over a 15 day time course. It appears from early inspection of the data, as if tetraplatin is less nephrotoxic, but that the toxicity is not dramatically less. In addition, the histopathologic distribution of the lesion is generally throughout the cortex, rather than being restricted to the corticomedullary junction, as is the case with cisplatin. We are continuing to evaluate our data so that we we will be able to draw more definite conclusions regarding the relative toxicity of this analog.

Publications:

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2. Litterst, C.L.: Cisplatinum: A review, with special reference to cellular and molecular interactions. Agents Actions 15: 520-525, 1985.

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6. Farris, F.F., King, F.G., Dedrick, R.L. and Litterst, C.L.: Physiological model for the pharmacokinetics of cis-dichlorodiammineplatinum (II) (DDP) in the tumored rat. J. Pharmacokin. Biopharm., in press.

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8. Kelley, J.A., Roth, J.S. and Litterst, C.L.: Gas chromatographic determination of hexamethylene bisacetamide in plasma and urine. Anal. Letters, in press.

9. Litterst, C.L., Smith, M., Smith, J., Uozumi, J. and Copley, M.: Sensitivity of urinary enzymes as indicators of renal toxicity of the anticancer drug cisplatinum. In Bianchi, C. and Blaufox, D.M. (Eds.): Newer Diagnostic Methods in Nephrology and Urology. Basel, Switzerland, Karger Publishing Co., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07120-06 LPET

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 cherecters 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 below the Principal investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. E. Gram	Pharmacologist	LPET, NCI
Others:	J. Goochee	Chemist	LPET, NCI
	L. K. Okine	Visiting Fellow	LPET, NCI

COOPERATING UNITS *(if any)*: National Heart, Lung and Blood Institute, NIH (M. C. Lowe)

LAB/BRANCH: Laboratory of Pharmacology and Experimental Therapeutics

SECTION: Biochemical Pharmacology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	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.)*

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. Accompanying the lung damage there was a significant impairment of pulmonary cytochrome P-450 linked monooxygenase activities. Simultaneous with these changes there was a paradoxical increase in certain of these activities in kidney; these increases were found to be the result of enzyme induction.

PHS 6040 (Rev. 1/84) GPO 914-918

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

1. In vivo distribution of [^{14}C] 1,1-dichloroethylene, covalent binding, and effects of drugs in the mouse. The distribution and covalent binding of a single dose of [1,2-^{14}C]1,1-dichloroethylene (DCE; 125 mg/kg, I.P.) were studied in male C57Bl/6N mice. Total radioactivity was distributed in whole homogenates of all tissues studied with peak levls occurring within 6 hours. Covalent binding of radioactive material peaked at 6-12 hours in all tissues and highest levels were found in kidney, liver and lung with smaller amounts in skeletal muscle, heart, spleen, and gut. Covalent binding in kidney, liver and lung fell to 50% of peak levels in about 4 days. Between 12 hours and 4 days after DCE administration, 70-100% of total radioactivity present in homogenates of kidney, liver and lung was covalently bound. The three tissues showed similar spread in total radioactivity in subcellular fractions 24 hr after exposure to DCE; most of the radioactivity was covalently bound (60-100% and distributed fairly uniformly with a slight tendency to concentrate in the mitochondrial fraction. Phenobarbital (PB) and 3-methylcholanthrene (3-MC) pretreatments increased the covalent binding in the liver and lung but had no effect in kidney. Piperonyl butoxide and SKF-525A decreased the covalent binding in liver and lung but the latter increased binding in the kidney while the former decreased it. Diethylmaleate administration increased the covalent binding (2- to 3-fold) in all three tissues as well as increasing lethal toxicity. These results are consistent with the view that DCE is metabolized to some reactive intermediate(s) which may be detoxified by conjugation with glutathione.

2. Effect of steroid pretreatment on the pulmonary damage produced by butylated hydroxytoluene in mice. The effects of the synthetic corticosteroid methylprednisolone (MP; 30 mg/kg, s.c. given twice daily for 3 days), on the pneumotoxic effects of a single dose of butylated hydroxytoluene (BHT; 400 mg/kg, i.p.) over a 10 day experimental period was investigated in male C57Bl/6N mice. BHT alone caused time-dependent alveolar hypercellularity, inflammatory infiltration, alveolar septal thickening with some degree of recovery by day 10. The pulmonary monoosygenase activities reflected the degree of alveolar damage and Clara cell abnormality with time; reductions in monooxygenase activities occurred and minimum levels (7-15% of control) were reached by day 5 and again a trend towards recovery by day 10. MP administered 0, 24 and 48 hr after BHT treatment partially protected mice from these effects of BHT in a distinctly time-dependent fashion; the degree of protection decreased as the time between BHT challenge and MP treatment increased. Although MP alone did not morphologically affect Clara and alveolar cells, it increased, decreased or had no effect on the monooxygenase activities. About 25% of the mice that received BHT alone died by day 5 and 50% by day 10. MP completely blocked the lethal effects of BHT by day 5 and reduced the deaths to between 15% and 25% by day 10. Interestingly, MP did not protect against the BHT-induced pulmonary fibrosis, measured as total lung hydroxyproline content, irrespective of the time between BHT challenge and MP treatment. MP alone did not cause any deaths nor increase lung hydroxyproline content.

Publications:

1. Trush, M.A., Reasor, M.J. and Van Dyke, K.: Oxidant-mediated electronic excitation of imipramine. Biochem. Pharmacol. 33: 3523-26, 1984.

2. Krijgsheld, K.R., Lowe, M.C., Mimnaugh, E.G., Trush, M.A., Ginsburg, E. and Gram, T.E.: Selective damage to nonciliated bronchiolar epithelial cells in relation to imapirment of pulmonary monooxygenases activities by 1,1-dichloroethylene in mice. Toxicol. Appl. Pharmacol. 74: 201-213, 1984.

3. Krijgsheld, K.R. and Gram, T.E.: Selective induction of renal microsomal cytochrome P-450-linked monooxygenases by 1,2-dichloroethylene in mice. Biochem. Pharmacol. 33: 1951-1956, 1984.

4. 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, 1985, pp. 421-470.

5. Okine, L.K., Goochee, J.M. and Gram, T.E.: Studies on the distribution and covalent binding of 1,1-dichloroethylene in the mouse: Effect of various pretreatments on covalent binding in vivo. Biochem. Pharmacol., in press.

6. Okine, L.K., Lowe, M.C., Mimnaugh, E.G., Goochee, J.M. and Gram, T.E.: Protection by methylprednisolone against butylated hydroxytoluene-induced pulmonary damage and impairment of microsomal monooxygenase activities in the mouse: lack of effect on fibrosis. Exp. Lung Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07122-05 LPET

PERIOD COVERED: October 1, 1984 to September 30, 1985

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

Biochemical and Pharmacological Studies with Oncolytic Nucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D. A. Cooney	Senior Investigator	LPET, NCI
Others:	Y. A. Wilson	Chemist	LPET, NCI
	M. Dalal	Microbiologist	LPET, NCI
	E. B. Gregory	Bio. Lab. Tech.	LPET, NCI
	G. Kang	Visiting Fellow	LPET, NCI
	V. E. Marquez	Visiting Scientist	LPET, NCI

COOPERATING UNITS *(If any)*

Laboratory of Biological Chemistry (R. Glazer and S. Arnold)

LAB/BRANCH: Laboratory of Pharmacology and Experimental Therapeutics

SECTION: Biochemical Pharmacology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

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

☐ (a1) Minors

☐ (a2) Interviews

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

Biochemical and Pharmacologic Studies with Arabinosyl-5-Azacytosine (Ara-AC)

Ara-AC is a hybrid nucleoside combining the structural elements of arabinosyl cytosine and 5-azacytidine. During the past year, systematic studies were carried out to determine the mechanisms by which tumor cells acquire resistance to this agent. Three models were adopted: 1) spontaneous resistance, exemplified by the transplantable colon carcinoma 38; 2) acquired resistance provoked by cultivation of P388 cells in the presence of incremental concentrations of Ara-AC over 100 generations; 3) resistance provoked by mutagenesis with MNNG. In all three cases, the resistance was accompanied by a fall, and in some cases a deletion of deoxycytidine kinase, the enzyme responsible for inaugurating the anabolism of the drug. In the case of the colon carcinoma 38, a second enzymatic factor was identified, namely an abundant endowment with a cytosolic nucleoside triphosphatase, which prevents the accumulation of adequate levels of the proximate antimetabolite, Ara-AC-triphosphate. These studies were next extended to a panel of cultured human tumor cells. In these lines there was a positive correlation ($r = 0.63$) between deoxycytidine kinase levels and the IC_{50} of Ara-AC. It is therefore proposed that measurement of this kinase might well be of prognostic value in patients scheduled to receive Ara-AC in its forthcoming Phase I trials.

Studies with Arabinosyl-5-Azacytosine (Ara-AC)

Ara-AC is a hybrid nucleoside, synthesized in the Division of Cancer Treatment and being readied for Phase I trials with a high priority. In last year's Report, studies on the mechanism of action of this drug were recapitulated: Ara-AC undergoes extensive anabolism to the 5'-triphosphate which in turn inhibits the synthesis of DNA, but is also incorporated into that macromolecule, where it undergoes ring-opening and engenders inhibition of methylation. During the present year an extensive series of experiments were conducted to determine the mechanism(s) by which tumor cells become resistant to Ara-AC. This study is rendered all the more relevant by virtue of the apparently facile emergence of resistance to Ara-C and 5-AC in patients receiving these agents. Three categories of resistance were examined: spontaneous or native resistance, exemplified by the colon carcinoma 38; resistance provoked by cultivation of P388 cells in the presence of incremental concentrations of Ara-AC over 100 transplant generations; and resistance provoked by exposure to MNNG followed by cloning in agarose. This procedure yielded 8 separate clones, all capable of growing normally in concentrations of the drug ~1000 times those tolerated by the parental strain. Resistance in all of aforementioned cases was found to be associated with a depression in or deletion of the specific activity of deoxycytidine kinase the enzyme which inaugurates the metabolism of Ara-AC. An identical mechanism is very frequently associated with resistance to Ara-C. Interestingly, a second factor contributing to resistance was uncovered in Colon Carcinoma 38: namely, a rich endowment with a cytosolic nucleotidase, which preferentially degrades nucleoside triphosphates. This activity would of course preclude the maintenance of effective levels of the putative proximate antimetabolite of Ara-AC, namely Ara-AC-triphosphate. Pursuant to these studies with rodent cells and tumors, a prospective examination was conducted of the relationship between the concentration of deoxycytidine kinase and the cytotoxicity of Ara-AC towards a panel of cultured human tumor cells. Although the correlation between these two parameters was positive ($r = 0.633$) it was not perfect. Nevertheless it was clear from the results that tumor cells deficient in deoxycytidine kinase were more refractory to Ara-AC than their well-endowed counterparts.

Publications:

1. Gebeyehu, G., Marquez, V.E., Van Cott, A., Cooney, D.A., Kelley, J.A., Jayaram, H.N., Ahluwalia, G.S., Dion, R.L., Wilson, Y.A. and Johns, D.G.: Ribavirin, tiazofurin, and selenazofurin: mononucleotides and nicotinamide adenine dinucleotide analogs. Synthesis, structure and interactions with IMP dihydrogenase. J. Med. Chem. 28: 99-105, 1985.

2. Tono-Oka, S., Tone, Y., Marquez, V.E., Cooney, D.A., Sekikawa, I. and Azuma, I.: Enzymatic synthesis and biochemical activity of various indazole adenine dinucleotides. Bulletin of the Chemical Society of Japan 58: 309-315, 1985.

3. Carney, D.N., Ahluwalia, G.S., Jayaram, H.N., Cooney, D.A. and Johns, D.G.: Relationships between the cytotoxicity of tiazofurin and its metabolism by cultured human lung cancer cells. J. Clin. Invest. 75: 175-182, 1985.

4. Cooney, D.A., Stergis, G., Jayaram, H.N.: Enzymes, Therapeutic. In Kirk, R.E. and Othmer, D.F. (Eds.): Concise Encyclopedia of Chemical Technology. New York, John Wiley & Sons, 1985, pp. 429-430.

5. Cooney, D. and Kutzmits, R.: Pharmacology of anticancer drugs. Cancer Treat. Rev., in press.

6. Tyagi, A.K. and Cooney, D.A.: Biochemical pharmacology, metabolism and mechanism of action of L-alanosine, a novel natural antitumor agent. Adv. Pharmac. Chemother. 20: 69-121, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07129-04 LPET

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Copper and Its Chelates in Cytotoxicity, Chemotherapy and Melanoma Promotion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Rabinovitz	Research Chemist	LPET, NCI
Others:	H. F. Pierson	PRAT Fellow	LPET, NCI
	J. M. Fisher	Chemist	LPET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology and Experimental Therapeutics

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

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 cytotoxicity of the copper chelate of the copper-specific chelating agent 2,9-dimethyl-1,10-phenanthroline to L1210 murine lymphoma cells in vitro had been shown to be due to its marked uptake by these cells. Such uptake was dependent upon the tetrahedral bidentate structure of the copper chelate and resulted in a small but significant in vivo chemotherapeutic activity. Accordingly, we had synthesized the corresponding halomethyl analogs in order to investigate their possible activity as copper-dependent, intercalating alkylating agents. These comprised the series: 2-chloromethyl-9-methyl-1,10-phenanthroline, NSC 382655; 2-bromomethyl-9-methyl-1,10-phenanthroline, NSC 382656; 2,9-bis-(chloromethyl)-1,10-phenanthroline; and 2,9-bis-(bromomethyl)-1,10-phenanthroline. Copper dependency for cytotoxicity was found only in the monohalomethyl compounds in the range 1 to 3 μM. They were submitted for chemotherapeutic evaluation with a procedure for formulation as cuprous chelates.

Copper chelates modulate B16 melanoma growth and metastasis in both a murine strain and copper chelate specific manner. Tumors failed to grow in CDF_1 and Swiss mice as subcutaneous transplants unless the mice were pretreated with copper chelates. Thus the cuprous chelate of neocuproine (2,9-dimethyl-1,10-phenanthroline) promoted testicular metastasis in NIH Swiss mice but inhibited them in CDF_1 mice, while the nitrilotriacetic acid cupric chelate permitted such metastases in both strains.

Objectives:

Our studies are directed toward understanding the basis of copper-mediated cytotoxicity and the sensitivity to copper and its chelates to tumor cells. This information is applied to the design of regimens and new agents for chemotherapy.

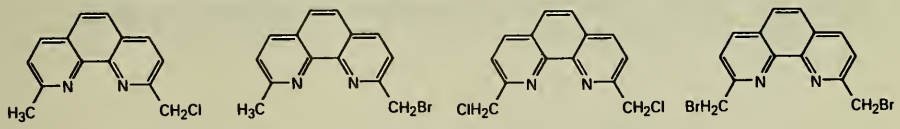
Methods Employed:

The principal methods employed involve techniques such as primary and established cell culture, viability estimates by clonal growth, cell size distribution and chemotherapeutic evaluation with tumor bearing mice. Studies with isotopically labelled components are also carried out in experiments designed to ascertain mechanisms of action. Information gained from such biochemical studies is applied to the design of new agents, a route for their synthesis is proposed and after preparation, the new compounds are evaluated for chemotherapeutic efficacy and postulated mechanisms of action.

Major Findings:

A. A New Class of Halomethylphenanthrolines as Potential Copper-Dependent, Chemotherapeutic Agents:

The copper-specific chelating agent, 2,9-dimethyl-1,10-phenanthroline had significant but limited chemotherapeutic activity against the P388 lymphoma when administered in vivo together with copper (Biochem. Pharmacol. 32: 362, 1983). Cytotoxicity was due to marked uptake of the copper chelate by the cells, which in turn was dependent upon the unique tetrahedral ring system formed only with the 2- and 9-dimethyl derivative. In order to exploit this property more fully, we had four members of a new class of 2,9-halomethylphenanthrolines synthesized as potential copper-dependent, DNA-intercalating, alkylating agents. These were prepared by Drs. Vinod Gupta and George Newkome of the Department of Chemistry, LSU, Baton Rouge, LA.



I II III IV

I 2-chloromethyl-9-methyl-1,10-phenanthroline; NSC 382655
II 2-bromomethyl-9-methyl-1,10-phenanthroline; NSC 382656
III 2,9-bis-(chloromethyl)-1,10-phenanthroline
IV 2,9-bis-(bromomethyl)-1,10-phenanthroline

All 4 componds formed bright yellow cuprous chelates and were found to be cytotoxic to P388 cells in primary culture in the range 1 to 3 μM. However, copper dependency for cytotoxicity was observed only in the monohalogenated compounds, and these were submitted for chemotherapeutic evaluation with a procedure for formulation as cuprous chelates.

B. Copper Chelates Promote Melanoma Tumorigenicity (by Herbert Pierson)

This study was aimed at clarifying the role of exogenous copper on B16 melanoma in vivo. Groups of BDF_1, DBA, CDF_1 and NIH Swiss mice were treated ip with either the cupric chelate or nitrilotriacetic acid (NTA: Cu^{+2}, 20:10 μmoles/kg) or the cuprous chelate of neocuproine (NC:Cu^{+1}, 10:5 μmoles/kg) every other day for 7 days prior to tumor inoculation and every other day for 15 days thereafter. Treatment with NC:Cu^{+1}: promoted tumor growth; increased body weight gain; stimulated tumor growth rate; inhibited tumor encapsulation; permitted tumor growth as unrestrained ascites; enhanced tumor pigmentation; and increased the final weight of tumors despite hastened host mortality. Tumors failed to grow in CDF_1 and NIH Swiss mice as sc transplants unless mice were pretreated with copper chelates. NTA:Cu^{+2} had little influence on metastasis of rapidly growing tumors (BDF1), partially inhibited pulmonary and testicular metastasis, but enhanced hepatic metastasis of slowly growing tumors (DBA); and permitted pulmonary, hepatic, and testicular metastasis in CDF1 and NIH Swiss mice. NC:C^{+1} enhanced pulmonary metastasis of rapidly growing tumors (BDF1); partially inhibited pulmonary metastasis of slowly growing tumors (DBA); inhibited testicular metastasis in CDF1 mice, and enhanced pulmonary, hepatic and testicular metastasis in NIH Swiss mice. These results suggest that exogenous copper modulates B16 melanoma growth and metastasis in both a murine strain and copper chelate-specific manner. Possible mechanisms include enhanced copper nutriture of tumors and inhibition of immune responses.

Publications:

1. Pierson, H.F.: Pharmacological perturbation of murine melanoma growth by copper chelates. Cancer Lett. 26: 221-233, 1985.

2. Pierson, H.F.: Administration of copper chelates enhances tumorgenicity of B16 melanoma in heterogenetic mice. Cancer Treat. Rep., in press.

3. Pierson, H.F. and Meadows, G.G.: Nutritional and pharmacological modulation of peroxidation in murine melanoma. J. Natl. Cancer Inst., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 07164-01 LPET

PERIOD COVERED

October 1, 1985 to September 30, 1985

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

Differential Competition between Metabolites and Cytotoxic Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Rabinovitz	Research Chemist	LPET, NCI
Others:	H. F. Pierson	PRAT Fellow	LPET, NCI
	J. M. Fisher	Chemist	LPET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pharmacology and Experimental Therapeutics

SECTION

Biochemical Pharmacology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.75	0.5	0.25

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.)*

Taurine mustard, N-bis-(2-chloroethyl)aminoethane sulfonic acid, had significant chemotherapeutic activity against the P388 and L1210 lymphocytic leukemias, and the pigmented and unpigmented B16 melanomas. Its cytotoxicity toward the P388 in primary culture (IC_{50} = 50 μM) was not reduced by a high taurine concentration (5 mM). Taurine also did not reduce its efficacy in vivo. However, in vivo, taurine protected against neurotoxicity, intestinal necrosis, pulmonary emboli formation and tail vein necrosis when administered i.v. with taurine mustard.

Objectives:

The principal objective of this ongoing series of studies is to evaluate the competitive interaction between a metabolite and a newly designed cytotoxic analog. Information obtained in this manner is applied to the tumor bearing animal in attempts to limit toxicity to the host.

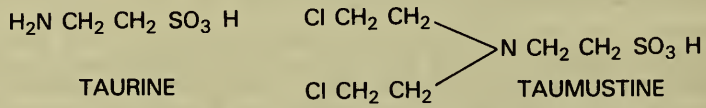
Methods Employed:

The principal methods employed involve techniques such as primary and established cell culture, viability estimates by clonal growth, cell size distribution and chemotherapeutic evaluation with tumor bearing mice. Studies with isotopically labelled components are also carried out in experiments designed to ascertain mechanisms of action. Information gained from such biochemical studies is applied to the design of new agents, a route for their synthesis is proposed and after preparation, the new compounds are evaluated for chemotherapeutic efficacy and postulated mechanisms of action.

Major Findings:

Taurine Protects Host Tissues from the Toxicity of Taurine Mustard:

The high intracellular concentration of taurine in tumors, its unique uptake characteristics, together with its suggested role as a displacer of histone and non-histone protein from DNA as a mechanism for its effect on cellular proliferation made an investigation of taurine mustard, N-bis(2-chloroethyl)aminoethanesulfonic acid (TM) of interest.



The antitumor activity of taurine mustard (taumustine) was evaluated in the murine P388 and L1210 lymphocytic leukemias, and the pigmented and nonpigmented B16 melanoma systems. Antitumor activity was found to be dependent upon the route of administration, treatment schedule and anatomical placement of tumor inoculum. Treatment with a single i.p. dose of taumustine (40 mg/kg) resulted in a 130% increase in survival for mice bearing P388 (i.p.), 93% increase in survival for mice bearing L1210 (i.p.), and about 80% increase in survival for mice bearing B16 melanoma (i.p.). Treatment with a single dose of taumustine increased the survival of mice bearing s.c. melanomas by 45% and decreased final tumor weight, but did not increase survival of mice bearing s.c. L1210 leukemia despite a reduction of final tumor weight. Repeated low doses (10 mg/kg) of taumustine resulted in 250% increase in survival for mice bearing P388 (i.p.), absence of ascites from day 4 onward, and the presence of pulmonary emboli from day 5 onward.

The inclusion of taurine (5 mM) in the culture medium of P388 cells in primary culture for 45 hr did not alter the cytotoxicity of taumustine (IC_{50} = 50 μM). However, pretreatment of the tumor bearing host with taurine (250 mg/kg) 30 min

before daily treatments with taumustine for up to 8 days did not interfere with antitumor activity (140-160% increased survival), promoted ascites formation independent of the number of treatments, and afforded protection against the formation of pulmonary emboli. Treatment of tumor-bearing mice with taurine also abrogated neurotoxicity, intestinal necrosis, pulmonary emboli formation, and tail vein necrosis due to the administration of a toxic dose of taumustine. The modulation by taurine of taumustine activity suggests that the combination of these agents may offer an advantage of selectivity and host protection during chemotherapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07165-01 LPET

PERIOD COVERED: October 1, 1985 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: The Sulfhydryl Group in Cancer Cell Growth and in Chemotherapy

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	M. Rabinovitz	Research Chemist	LPET, NCI
Other:	H. F. Pierson	PRAT Fellow	LPET, NCI
	J. M. Fisher	Chemist	LPET, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH: Laboratory of Pharmacology and Experimental Therapeutics

SECTION: Biochemical Pharmacology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	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.)*

Many tumors in primary culture, both murine and human, cannot utilize cystine as a source of cysteine for growth. Attempts were therefore made to maintain an oxidative stress in vivo which would convert available cysteine to cystine and thus inhibit tumor growth. The mixture of hydroxocobalamin (vit. B-12) and ascorbate were very efective in oxidizing cysteine in vitro and were shown to increase the life span of P388 lymphoma-bearing mice by 70 percent.

Cells which will not grow with cystine as a source of cysteine require mercapto-ethanol or its oxidized form, hydroxyethyl disulfide, as a growth factor. These facilitate the uptake by the cell of the cysteine moiety as the mixed disulfice. A similar interaction with inverse purpose, the promotion of cytotoxicity of homo-cystine, selenocystine and selenocystamine by dimethyl disulfide was discovered. This reaction increased the cytotoxicity of selenocystamine in vitro by two orders of magnitude while reducing its host toxicity in vivo.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

These studies are directed toward understanding the critical role of mercaptoethanol in support of growth of tumor cells in primary culture and exploiting this role to chemotherapeutic advantage.

Methods Employed:

The principal methods employed involve techniques such as primary and established cell culture, viability estimates by clonal growth, cell size distribution and chemotherapeutic evaluation with tumor bearing mice. Studies with isotopically labelled components are also carried out in experiments designed to ascertain mechanisms of action. Information gained from such biochemical studies is applied to the design of new agents, a route for their synthesis is proposed and after preparation, the new compounds are evaluated for chemotherapeutic efficacy and postulated mechanisms of action.

Major Findings:

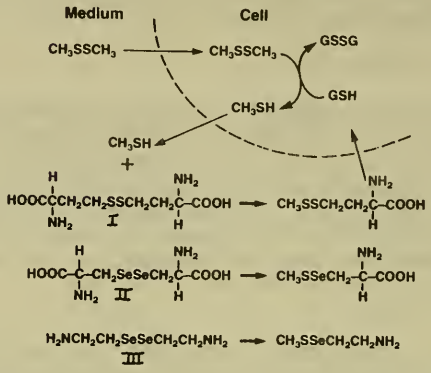
1. Depletion of Extracellular Cysteine with Hydroxocobalamin and Ascorbate in Experimental Cancer Chemotherapy:

Treatment of mice bearing P388 leukemia with combined hydroxocobalamin (0.10 mmole/kg) and sodium ascorbate (1.0 mmoles/kg) for 10 consecutive days resulted in 70% increase in survival. Treatment with dehydroascorbate (1.0 mmole/kg) produced similar increased survival, but treatment for 15 days did not improve survival. P388 cells incubated in primary culture with cystine as a source of cysteine require exogenous thiols or disulfides for growth; and also grow with cysteine, but only when trace copper is sequestered with bathocuproine sulfonate, a copper specific chelate which prevents copper catalyzed oxidation of cysteine to cystine. Cells grew in medium preincubated for 8 hr with cysteine and bathocuproine sulfonate, but lysed when the preincubated medium included hydroxocobalamin (10 μM) and sodium ascorbate (100 μM). This defect was corrected by resupplementation with cysteine or cystine and bishydroxyethyl disulfide (25 μM) after addition of the cells. Further support for cysteine depletion in therapy is obtained by therapeutic interference with 4-thiamethionine (25.0 μmoles/kg). This mixed disulfide of cysteine and methyl mercaptan enters cells as a methionine analog, is reduced to supply cysteine, and thus withstands the oxidative environment that limits utilization of cysteine as a nutrient. Ultimate failure of therapy may be due to increased availability of cysteine produced by host tissues, in particular, infiltrating macrophages.

2. Adduct Formation in Selective Toxicity: Methylthio-capping of Selenocysteamine and its Potentiation of Antitumor Activity and Reduction of Host Toxicity

Low molecular weight sulfhydryl compounds or disulfides like mercaptoethanol or hydroxyethyl disulfide are known to be required for growth of murine lymphomas and human tumors in primary cell culture. This is due to the formation and facilitated transport of mixed disulfides of cysteine and low molecular weight sulfhydryl compounds. An analogous reaction between mercaptans and diselenides or selenols and disulfides forms unstable thioselenates. We have found that dimethyl disulfide was the most effective of several mercaptans and disulfides

for potentiating the cytotoxicity of homocystine I, selenocystine II, and selenocystamine III, in culture. A proposed mechanism by which dimethyl disulfoxide increases the cellular uptake is indicated below:



Cytotoxicity of all 3 compounds was accompanied by marked cell swelling and lysis. Such lysis was dependent on unbalanced growth, for homocystine treated cells did not lyse in the absence of methionine. High concentrations of leucine (5 mM) partially prevented the cytotoxicity of the mixture dimethyl disulfide and selenocystine, indicating that the methylthio-adduct of selenocysteine entered cells via the leucine transport system. However, both homocystine and selenocystine were too insoluble to formulate for in vivo experiments and our attention was therefore direct toward selenocystamine, a water soluble compound.

Dimethyl disulfide markedly increased the cytotoxicity of selenocystamine by 2 orders of magnitude (40 μM to 0.5 μM for 50% inhibition of growth in 45 hr). When mice were injected ip with 22 μmoles/kg of selenocystamine on Day 0, all were dead on Day 4. If dimethyl disulfide (500 μmoles/kg) was either mixed with selenocystamine or injected immediately prior to it, the mice survived treatment for at least 21 days afterwards, suggesting that methylthio-capping of selenocysteamine abrogated host toxicity. Such combined treatment may be of value in chemotherapy.

Publications:

1. Mohindru, A., Fisher, J.M. and Rabinovitz, M.: Endogenous copper is cytotoxic to a lymphoma in primary culture which requires thiols for growth. Experientia, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07166-01 LPET

PERIOD COVERED: October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Biochemical and Pharmacologic Studies with Oncolytic Barbituric Acid Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

P.I.:	D. A. Cooney	Senior Investigator	LPET, NCI
Others:	Y. A. Wilson	Chemist	LPET, NCI
	M. Dalal	Microbiologist	LPET, NCI
	E. B. Gregory	Bio. Lab. Tech.	LPET, NCI
	G. Kang	Visiting Fellow	LPET, NCI

COOPERATING UNITS *(if any)*
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Laboratory of Experimental Therapeutics and Metabolism (J. McMahon)
Clinical Pharmacology Branch, COP (B. Sinha)

LAB/BRANCH: Laboratory of Pharmacology and Experimental Therapeutics

SECTION: Molecular Pharmacology Section

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	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.)*

Biochemical and Pharmacologic Studies with Oncoloytic Barbituric Acid Derivatives

Merbarone is a hybrid molecule, consisting of thiobarbituric acid conjoined to aniline in an amide linkage; the drug is curative of the L1210 leukemia. In last year's Annual Report it was documented that merbarone promptly arrested DNA synthesis in cultured L1210 cells. In the present year, the ability of merbarone to produce damage to DNA was examined. These studies used cultured L1210 cells as their principal tool. Exposure of these cells to an IC_{50} concentration of the drug for 16 hours resulted in a highly significant rate of single-strand breaks. These breaks did not, however, appear to be protein-associated. Approximately 24 hours were required for the repair of 50% of the SSB's. When merbarone was incubated with hepatic microsomes and NADPH, ESR signals suggestive of oxygen-centered radicals were generated. As a working hypothesis, it is suggested that such radicals may be mediating the DNA damage described here.

Initial Mechanistic Studies with Merbarone (NSC 336628):

The structure of merbarone [5-(N-phenylcarboxamido)-2-thiobarbituric acid (NSC 336628) is shown in Fig. 1. Originally synthesized as a potential herbicide, the compound was discovered to exhibit curative activity against L1210 leukemia, when administered to mice by either the intraperitoneal or oral routes. Because of this activity, merbarone is now being readied for clinical trials. As an adjunct to those trials, our laboratory has instituted studies into the mechanism of action of this structurally novel molecule. L1210 cells, cultivated in RPMI 1630 medium, served as the principal experimental tools for our studies. Exposure of logarithmically growing cultures of these murine lymphoblasts to graduated concentrations of merbarone (1-100 μM, dissolved in DMSO) resulted in progressive inhibition of proliferation. The median inhibitory concentration (IC_{50}) was 10 μM (range 9-13 μM). Cloning experiments revealed that a twenty-four hour exposure to 10 μM merbarone produced a 0.5 log decrease in viability, higher concentrations being progressively more cytocidal. Preliminary pharmacokinetic studies established that a single intraperitoneal injection of [^{14}C]merbarone, at its optimal therapeutic dose (50 mg/kg) produced plasma concentrations of drug-derived radioactivity which exceeded or approximated 10 μM for ~4 hr. This result establishes that the concentrations of merbarone used *in vitro* can be achieved *in vivo*.

Flow cytometry revealed that a 16 hr exposure of L1210 cells to merbarone (10 μM) markedly slowed progression of cells from S phase. Parallel studies of the influence of merbarone on macromolecular syntheses revealed that a 2 hr exposure to an IC_{50} concentration (10 μM) of the drug failed to alter nucleic acid or protein synthesis, but that a 24 hr exposure reduced the rate of DNA synthesis by 30-50%. High concentrations of merbarone (>IC_{99}) promptly and totally blocked the incorporation of L-valine, uridine and thymidine into macromolecules. Transformation of human lymphocytes in the presence of 20 μM merbarone did not lead to prominent chromosomal aberrations. Mechanistic studies with merbarone were initially guided by its bipartite structure - a molecular conjugate of thiobarbituric acid and aniline, joined in amide linkage. Thiobarbituric acid has two major biologically significant properties: its reactivity with dialdehydes, notably malonaldehyde, and its ability to inhibit the membrane enzyme, γ-glutamyl transpeptidase. Merbarone, tested at 100 μM under published conditions failed to function as a substrate or inhibitor in either system. Aniline is known to react with erythrocytes producing methemoglobinemia in susceptible subjects. Merbarone incubated *in vitro* with human red cells (10-100 μM) failed to produce methemoglobin. Parenthetically, it is relevant that merbarone was also nitrobenzyl pyridine-negative and so, is unlikely to function as an alkylating species - at least in its native state. The drug also was incapable of inhibiting the respiration of intact L1210 cells to a significant degree in vitro (at concentrations up to 50 μM) and so is unlikely to function as a straightforward respiratory inhibitor.

The pyrimidine moiety of merbarone prompted studies of its possible role as an antimetabolite. However, exposure of L1210 cells to 10 μM drug for 24 hr failed to engender significant perturbation of their nucleotide or amino acid pools; a 5 hr exposure to 5 and 10 μM merbarone also was without influence on the cellular content of PRPP. In addition, none of the customary preformed purines or pyrimidines (singly, and in combination) or amino acids, tested at

their maximally tolerated concentrations, proved capable of overcoming merbarone's cytotoxicity, thus making it unlikely that the drug was behaving as a classical antimetabolite.

Attempts to determine whether merbarone might bind to DNA were complicated by its avid affinity for conventional cellulose dialysis tubing, and a variety of other polymeric materials. When nucleic acids were extracted from L1210 cells exposed to 10 μM [^{14}C]merbarone for 16 hr, using SDS phenol extraction, only negligible radioactivity was associated with these macromolecules. The drug also appeared not to displace the intercalating compound, ethidium bromide, from calf thymus DNA as adjudged by fluorescence spectrometry. However, when L1210 cells were exposed to merbarone (10-50 μM) for 16-24 hr and their DNA examined by alkaline elution. Significant numbers of dose-related single strand breaks (SSB's) were demonstrable. SSBs were produced in both [^{14}C]-thymidine prelabeled cells and in cells exposed to [^{14}]thymidine and merbarone simultaneously, although the latter protocol resulted in higher single strand break frequencies. Proteinase-K did not materially alter the elution pattern of DNA damaged by merbarone, suggesting that the SSB's are not protein-associated. Moreover, initial experiments demonstrated no formation of DNA-protein crosslinks by this compound.

In order to examine the rate at which cells repaired the merbarone-induced lesions in their DNA, alkaline elution analyses were conducted at representative time-points after removal of the drug. These experiments demonstrated that about 12 hr were required for the repair of 50% of these lesions. When merbarone was incubated with hepatic microsomes and NADH, ESR signals suggestive of the presence of oxygen-centered radicals were generated.

Although it is not clear from these experiments whether merbarone itself, or a metabolite, produces the DNA damage observed by alkaline elution, this damage does seem to be implicated in the therapeutic action of the drug inasmuch as it is seen at comparatively low concentrations of merbarone (from the IC_{20} upward). It will remain for future studies to pursue the lines of investigation suggested by the data reported herein.

ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

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 of 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 and 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. More than 100 isolates of HTLV-III have been obtained from patients with acquired immune deficiency syndrome (AIDS) and pre-AIDS.

2. HTLV-III has been isolated from semen or saliva of AIDS patients.

3. Seroepidemiological studies with HTLV-III show that 95% of the AIDS and pre-AIDS patients and approximately 45% of healthy homosexuals carry HTLV-III antibodies.

4. The sera from AIDS and pre-AIDS patients precipitates proteins of 41,000, 120,000 and 160,000 daltons.

5. Serological evidence for high prevalence (65%) of HTLV-III antibody in Ugandan children surveyed in 1972.

6. Development of an assay for detection of neutralizing antibodies to HTLV-III in sera of individuals exposed to the virus.

7. Demonstration of heterosexual transmission of HTLV-III by serologic detection of antibodies in sexual partners of AIDS and ARC cases in New York.

8. Seroepidemiological studies show that populations at risk for development of AIDS include Canadian and Japanese hemophiliacs, Haitian immigrants to New Guinea, Zairians, Rwandese, and male prostitutes in Singapore.

9. A monoclonal antibody against a 41,000 dalton protein induced by HTLV-III infection has been prepared and characterized.

10. Demonstration of an 18% prevalence of HTLV-III antibodies among black drug users in New York.

11. HTLV-I antibodies have been found in sickle cell anemia patients in Martinique, Venezuela, several villages of Alaskan Aleuts and Swedish Lapps.

12. Studies on the development of an animal model indicate chimpanzees to be a suitable animal. Seroconversion and HTLV-III isolation was demonstrated in chimps infected with HTLV-III.

13. Development of an in situ hybridization technique to detect HTLV-III sequences in several tissues, including brain, from patients with AIDS and ARC.

14. Several drugs, including foscarnet, cis-plantinum and lapachone derivatives have been shown to inhibit HTLV-III replication.

15. Molecularly cloned and sequenced several HTLV-III isolates. Showed HTLV-III to be similar to LAV and ARV.

16. Determined the splice acceptor site for the pX gene of HTLV-I and sequenced the pX region of HTLV-I_b.

17. Developed a biologically active plasmid clone by using molecular clones of HTLV-III and a protoplast fusion technique.

18. Determined the nucleotide sequence of the envelope gene of HTLV-III (RF).

19. Demonstration of trans-acting transcriptional activation for HTLV-III.

20. Demonstration of HTLV-III to visna virus.

21. Demonstration of HTLV-III infection in brains of AIDS patients with encephalopathy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 CM 06117-13 LTCB

PERIOD COVERED
October 1, 1984 to September 30, 1985

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 below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert C. Gallo	Chief	LTCB NCI
Marvin S. Reitz	Chemist	LTCB NCI
Prem S. Sarin	Chemist	LTCB NCI
W. Carl Saxinger	Microbiologist	LTCB NCI
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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
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
56	33	23

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

Suresh Arya	Cancer Expert	LTCB NCI
S. Zaki Salahuddin	Cancer Expert	LTCB NCI
Ann Sliski-Mark	Cancer Expert	LTCB NCI
Suzanne Gartner	Staff Fellow	LTCB NCI
Mary Harper	Staff Fellow	LTCB NCI
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Genoveffa Franchini	Visiting Associate	LTCB NCI
Mikulas Popovic	Visiting Scientist	LTCB NCI
Anna Aldovini	Visiting Fellow	LTCB NCI
Chan Guo	Visiting Fellow	LTCB NCI
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Enrico Callalti	Guest Researcher	LTCB NCI
Sandra Colombini	Guest Researcher	LTCB NCI
Amanda Fisher	Guest Researcher	LTCB NCI
Corrado Gurgo	Guest Researcher	LTCB NCI
Shuji Nakamura	Guest Researcher	LTCB NCI
Jorg Schupbach	Guest Researcher	LTCB NCI
Bruno Starcich	Guest Researcher	LTCB NCI
Petr Stockbauer	Guest Researcher	LTCB NCI
George Shaw	IPA	LTCB NCI

COOPERATING UNITS

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Project 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.

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.

9. 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.

10. The distribution of HTLV in human T cell leukemia patients (HTLV-I, HTLV-II) and patients with acquired immune deficiency syndrome (AIDS) (HTLV-III) from various parts of the world is being actively pursued.

Major Findings

1. More than 100 isolates of HTLV-III have been obtained from patients with acquired immune deficiency syndrome (AIDS) and pre-AIDS.

2. HTLV-III has been isolated from semen or saliva of AIDS patients.

3. Seroepidemiological studies with HTLV-III show that 95% of the AIDS and pre-AIDS patients and approximately 45% of healthy homosexuals carry HTLV-III antibodies.

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19. Demonstration of trans-acting transcriptional activation for HTLV-III.

20. Demonstration of HTLV-III to visna virus.

21. Demonstration of HTLV-III infection in brains of AIDS patients with encephalopathy.

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 cloning and 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 fluorescence activated cell sorter (FACS), ficoll-hypaque gradients, sucrose density gradients, free flow electrophoresis and centrifugal elutriation. (Lancet 1: 508-509, 1976).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07148-02 LTCB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cellular Biological Studies on T-Cell Malignancies and Lymphomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Prem S. Sarin	Chemist	LTCB NCI
S. Zaki Salahuddin	Cancer Expert	LTCB NCI
Suzanne Gartner	Staff Fellow	LTCB NCI
Mikulas Popovic	Visiting Scientist	LTCB NCI
Jorg Jendis	Visiting Fellow	LTCB NCI
Yoshitaka Taguchi	Visiting Fellow	LTCB NCI
Shuji Nakamura	Guest Researcher	LTCB NCI
Petr Stockbauer	Guest Researcher	LTCB NCI

COOPERATING UNITS *(if any)*
Robin Weiss, Imperial Cancer Research Fund, London, England; Bart Haynes, Duke University; Ken McCredie, Anderson Hospital and Tumor Institute; Myron Essex, Harvard University.

LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
15	8	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.)*

The cell biology studies have been focused on the role of human T lymphotropic retroviruses on human T cell malignancies and acquired immune deficiency syndrome (AIDS). More than 100 isolates of HTLV-I have been obtained from patients with adult T cell leukemia-lymphoma, and over 100 isolates have also been obtained from patients with AIDS and pre-AIDS. HTLV-I has been shown to be a transforming virus whereas HTLV-III is cytopathic. Both HTLV-I and HTLV-III have specificity for OKT4 positive T helper cells. HTLV-III has been transmitted to a cell line which is productively infected with HTLV-III thus allowing the production of large quantities of virus for cell biology, molecular biological and biochemical studies. HTLV-III has also been isolated from saliva or semen from AIDS or pre-AIDS patients. HTLV-III isolates obtained from different patients show some genetic variations. An HTLV-III from a Haitian AIDS patient has been found to be the most divergent compared with other HTLV-III isolates. The virus probably attaches to the T cells through the OKT4 receptor since treatment of the virus with anti-T4 blocks infection of the receipient cells. Drugs that block HTLV-III replication are being tested in in vitro system. Preliminary studies indicate foscarnet, cis platinum and lapachone derivates may be useful in treatment of patients with AIDS or pre-AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07149-02 LTCB

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Biological Studies on HTLV and Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Flossie Wong-Staal	Microbiologist	LTCB NCI
Lee Ratner	Clinical Associate	LTCB NCI
Suresh Arya	Cancer Expert	LTCB NCI
Marvin Reitz	Cancer Expert	LTCB NCI
Mary Harper	Staff Fellow	LTCB NCI
Genoveffa Franchini	Visiting Associate	LTCB NCI
Anna Aldovini	Visiting Fellow	LTCB NCI
Chan Guo	Visiting Fellow	LTCB NCI

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Tumor Cell Biology

SECTION
Molecular Genetics of Hematopoietic Cells

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
22	15	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.)*

Studies on human retroviruses and oncogenes have been pursued with particular emphasis on their role in human disease. Two subgroups of a human T cell leukemia virus, designated HTLV-I and HTLV-II, have the unique capacity to transform human T cells in vitro, leading to cloned cell populations. Molecular cloning and comparative analysis of the genomes of HTLV-I and HTLV-II, revealed sequence conservation throughout, but particularly in a coding region designated pX and an enhancer sequences in the viral LTR. These results have direct relevance in the possible mechanism or transformation by these viruses. Recently HTLV-III has been postulated to be the etiologic agent of acquired immune deficiency syndrome. We have molecularly cloned and sequenced the HTLV-III genomes. A comparative analysis of the multiple HTLV-III isolats has shown divergence in the env gene. HTLV-III has been found to be similar to LAV or ARV and shows sequence homology to visna virus. HTLV-III infection has also been found in brain tissues of AIDS patients with encephalopathy. This was made possible by the recently developed in situ hybridization technique. It is now possible to express various HTLV-III proteins in prokaryotic systems and show transfection of biologically active HTLV-III DNA and demonstration of cytopathic effect.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Hong-Guang Guo	Visiting Fellow	LTCB NCI
Beatrice Hahn	Visiting Fellow	LTCB NCI
Ruth Jarrett	Visiting Fellow	LTCB NCI
Takashi Okamoto	Visiting Fellow	LTCB NCI
Amanda Fisher	Guest Researcher	LTCB NCI
Bruno Starcich	Guest Researcher	LTCB NCI
George Shaw	IPA	LTCB NCI

COOPERATING UNITS

Anderson Hospital and Tumor Institute; Bill Haseltine, Harvard University; Takis Papas, NCI-FCRF; Volker Erfle, Munich.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 07150-02 LTCB

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Seroepidemiological Studies on Human T-Lymphotropic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Prem S. Sarin	Chemist	LTCB NCI
W. Carl Saxinger	Microbiologist	LTCB NCI
Marjorie Robert-Guroff	Staff Fellow	LTCB NCI
Jorg Schupbach	Guest Researcher	LTCB NCI

COOPERATING UNITS *(if any)*

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LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION

Hematopoietic Cell Biochemistry and Immunology

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	4	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.)*

The worldwide distribution of HTLV infection, the mechanism of its transmission and its role in various types of T cell malignancies and patients with acquired immune deficiency syndrome (AIDS) and pre-AIDS has been extensively studied. A highly sensitive ELISA technique has been developed and extensively used for detection of HTLV infection. Seroepidemiological studies on the distribution of HTLV-III show that 95% of the AIDS and pre-AIDS patients, and approximately 45% of healthy homosexuals carry HTLV-III antibodies. High incidence (65%) of HTLV-III antibodies has also been found in sera of Ugandan children collected in 1972. Detection of HTLV-III antibodies in sexual partners of AIDS and ARC cases in New York suggest heterosexual transmission of HTLV-III. Other seroepidemiological studies show that populations at risk for development of AIDS include Canadian and Japanese hemophiliacs, Haitian immigrants to New Guinea, Zairians, Rwandese and male prostitutes in Singapore. The sera from AIDS and pre-AIDS patients has been shown to precipitate HTLV-III envelope proteins of 41,000, 120,000 and 160,000 daltons. HTLV-III inoculations into chimpanzees show that these animals seroconvert and virus has been reisolated from their peripheral blood indicating infection of these animals. None of these animals have so far developed AIDS-like syndrome.

PHS 6040 (Rev. 1/84) GPO 914-918

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1984 to September 30, 1985

The Laboratory of Molecular Pharmacology is carrying out studies in 3 major areas: (1) studies of the molecular mechanisms of action of alkylating agents such as chloroethylnitrosoureas and nitrogen mustards as well as platinum complexes; (2) studies of topoisomerase II as a target of action of DNA intercalating agents and epipodophyllotoxins; (3) studies of the regulation of chromosomal protein biosynthesis in relation to the control of cell proliferation. A major goal is to develop strategies for the selective killing of particular human tumor cell types.

DNA Chloroethylating Agents

A major continuing aim of the Laboratory has been to elucidate the mechanisms of the extraordinary activity of the chloroethylnitrosoureas (ClEtNUs) against a broad spectrum of murine tumors, and to work towards the optimized application of this mechanism to the treatment of human cancer. We had previously obtained evidence for a selective toxic mechanism against some human tumor cell strains that is based on the chemical addition of chloroethyl groups to guanine-O6 positions on DNA. The sensitive tumor cells had been reported to be deficient in the activity of an enzyme that removes alkyl groups, including chloroethyl groups, from guanine-O6 positions thus repairing these DNA lesions. Our data had suggested that the enhanced cell killing in the sensitive tumor cells was due to spontaneous chemical conversion of unrepaired O6-chloroethylguanines to possibly lethal interstrand crosslinks. These conclusions have now been supported by data from other laboratories. This selective cytotoxic mechanism is an attractive basis for cancer chemotherapy because we know the chemical lesion that probably is responsible for the selective killing of tumor cells, and a biochemical test can be devised to determine whether a given tumor has the required sensitivity characteristic: low guanine-O6-alkyltransferase activity.

The ClEtNU drugs used in clinical chemotherapy (BCNU, CCNU and MeCCNU) produce diverse chemical reactions with macromolecules in cells, and most of these reactions probably do not contribute to any therapeutic result. Our strategy is to reduce or eliminate unnecessary chemical reactivities by identifying other chloroethylating compounds that retain the high antitumor activities but lack some of the chemical side reactions.

We had previously focussed our attention on the carbamoylation reactions of CCNU and BCNU. We had presented evidence that carbamoylation reactions do not contribute to antitumor activity, but do interfere with the selective action against human tumor cells, probably on the basis of an observed inhibition of the ligase reaction in DNA nucleotide excision repair. We therefore argued for the development of a known non-carbamoylating analog to replace CCNU in the clinic.

At the same time, we proposed the development of a new type of chloroethylating agent, 2-chloroethyl(methanesulfonyl)methanesulfonate ("ClEtSoSo") which had recently been prepared at the Southern Research Institute and which was found to equal the ClEtNUs in its action against murine tumors. We reported last year that ClEtSoSo is similar to ClEtNUs in its crosslinking effects on DNA in human tumor cells and in its selective action against cells deficient in guanine-O6-alkyltransferase. These results strongly indicated that ClEtSoSo is a new chemical class of chloroethylating agent.

From its chemistry, it was clear that ClEtSoSo cannot carbamoylate. In addition, it seemed possible that ClEtSoSo would cause much less hydroxylation, an unnecessary side reaction that ClEtNUs prominently produce.

Our work during the current year addressed the questions: (1) whether ClEtSoSo does in fact exhibit the anticipated lower hydroxyethylation/chloroethylation ratio relative to ClEtNUs; (2) whether ClEtSoSo differs from ClEtNUs in regard to the base-sequence selectivity of its reactions with DNA.

Chloroethyl and hydroxyethyl adducts of guanine in DNA were isolated by means of HPLC and the chemical structures were verified by mass spectrometry in collaboration with Dr. John Strong, Laboratory of Medicinal Chemistry & Biology, DCT. It was found that, whereas a ClEtNU produced somewhat more 7-hydroxyethylguanine than 7-chloroethylguanine, ClEtSoSo produced 7-chloroethylguanine but no detectable hydroxyethyl products. Thus ClEtSoSo lacks two prominent unnecessary side-reactions of ClEtNUs: carbamoylation and hydroxyethylation.

The base-sequence selectivity for reaction of these compounds at guanine-N7 positions of DNA was determined by minor modifications of standard DNA sequencing methods. ClEtNUs were found to react with markedly different intensities with different guanines in a DNA fragment. Of particular interest was the finding of disproportionately strong reactions at runs of 4 or more G's. This suggests that ClEtNUs may react with strong preference for G-rich regions of DNA strands.

ClEtSoSo, on the other hand, exhibited little or no selectivity among different G's. This however refers to the guanine-N7 position whereas the major cytotoxic reaction more likely is at guanine-O6. Guanine-O6 adducts unfortunately cannot be detected by standard DNA sequencing techniques. We plan to study possible procedures that would allow this determination. We plan then to study modifications of the ClEtSoSo structure that may alter the base sequence selectivity, as well as the selectivity between the O6 and N7-guanine positions.

Studies were also carried out on human guanine-O6-alkyltransferase to improve the methods of isolation and assay. In addition, attempts were made to transfer this enzyme activity by means of purified DNA, as part of a continuing effort to isolate the gene and to determine the genetic cause of the deficiency in some human tumor.

A collaborative project with the Surgical Neurology Branch, NINCDS, is under way to test for a correlation between clinical response of malignant gliomas treated with aziridinylbenzoquinone, cisplatin or BCNU and the DNA interstrand cross-linking response to these drugs of cell lines derived from the tumors.

DNA Base Sequence Selectivity for Alkylation by Nitrogen Mustards

The nitrogen mustards are the oldest class of anti-cancer drugs and are still among the most effective available clinical drugs. Although large numbers of active nitrogen mustards were prepared in the 1950's and 1960's, there was during that time period no adequate molecular basis for optimizing the action of this class of compounds. We have during the past year initiated studies to apply modern techniques and concepts of DNA chemistry to this problem.

Current information, much of it from this Laboratory, indicates that the major cytotoxic lesions produced by nitrogen mustards are covalent crosslinks between the N7 positions of 2 guanine residues located on opposite DNA strands or adjacent to each other on the same strand. This suggeted that GC-rich regions in DNA may constitute hot-spots for alkylation and crosslinking by these drugs. We searched the DNA sequence data base to determine where in the human genome highly GC-rich regions may occur. Included among the most GC-rich regions were regions in some oncogenes, most notably in the 5' flank of H-ras. We therefore focussed our studies on a pBR plasmid containing a human H-ras insert.

After investigating several possible procedures for localizing DNA interstrand crosslinks in a DNA sequence, we concluded that this difficult problem is best deferred, and we concentrated our studies on guanine-N7 alkylations which are easily quantitated by standard DNA sequencing techniques and which are pre-requisite for the formation of both inter and intrastrand crosslinks.

The major findings to date are the following: (1) there are marked differences in reactivity among guanines in a DNA sequence; (2) the differences are not markedly dependent on the solvent conditions of reactions; (3) the guanine selectivity pattern is different for different nitrogen mustards; (4) the potential interstrand crosslinking sites -- e.g. 5'-GC-3' -- show relatively low reactivity for HN2, melphalan and spirohydantoin mustard; (5) these sites however show a markedly enhanced reaction with uracil mustard; (6) runs of G's (potential sites of intra-strand crosslinking) exhibited disproportionately high reactivity with most nitrogen mustards; (7) quinacrine mustard exhibited a uniquely different pattern of reactions.

We are beginning now to analyse the reaction intensity patterns quantitatively by means of densitometry and computer analysis. We will attempt to identify the elements of DNA sequence and structure that determine reactivity at specific sites. Molecular graphics systems will be utilized to formulate hypotheses on the basis of which nitrogen mustard structures with new or enhanced DNA sequence preferences could be synthesized.

Topoisomerase II as Target of Anticancer Drug Action

On the basis of previous work in this Laboratory, we had hypothesized an action of DNA intercalating agents on a DNA topoisomerase enzyme. This hypothesis was formulated as an explanation for the observed formation of protein-associated DNA strand breaks in mammalian cells treated with several DNA intercalating drugs, including m-AMSA (amsacrine), adriamycin, 5-iminodaunorubicin, ellipticine and 9-hydroxy-1-methylellipticinium. We had developed the methodology and the conceptual framework for the analysis of these DNA effects in mammalian

cells. Recent work in several laboratories has confirmed and extended this work, and another class of drugs, the epipodophyllotoxins, has been found to produce similar effects.

As reported in the previous Annual Report, we had isolated a fraction from cell nuclei that stimulated the intercalator-dependent production of protein-associated DNA strand breaks. In the meantime Dr. Leroy Liu and his coworkers at Johns Hopkins University, who had been studying purified mammalian topoisomerase II, made anologous observations on the effects of DNA intercalating agents and epipodophyllotoxins. Our active fraction from cell nuclei had the properties expected for topoisomerase II. Thus there is now strong evidence that the analysis of protein-associated strand breaks in mammalian cells that we had developed does in fact measure an effect on one or more topoisomerase enzymes.

During the current year we have investigated several aspects of the mechanisms of the drug effects on topoisomerase II and the consequent effects on cells.

Studies of a multi-drug resistant Chinese hamster cell line disclosed a reduced protein-associated strand break response to intercalators. In view of the possibility that this cell line might have developed several independent resistance mechanisms, an attempt was made to transfer the resistance to mouse 3T3 cells by means of DNA from the resistant cells. Two resistant clones of 3T3 cells have been obtained which show reduced protein-associated strand break responses and which contain Chinese hamster DNA. Our objectives now are to verify by repeated DNA transfer experiments that a dominant resistance gene has been identified and to determine its relationship to topoisomerase II.

We have found that the activity of topoisomerase II measured by the protein-associated strand break response is dependent on the proliferation state of the cells. In non-proliferating 3T3 cells, this activity was much less than in proliferating cells. When quiescent cells were stimulated to proliferate, the topoisomerase II response increased at about the time of the first wave of DNA synthesis, but did not then oscillate in phase with the cell cycle. Thus the observed alteration in topoisomerase II activity appears to be related to the proliferation state of the cells and not to phase of the cell cycle.

The epipodophyllotoxins (etoposide and teniposide) were studied. The formation of protein-associated strand breaks was verified in several cell lines and this was the only DNA effect detected in the pharmacologically reasonable dose range. The actions of these drugs on topooisomerase II were confirmed using enzyme purified from L1210 cells.

Regulation of Chromosomal Protein Synthesis and Control of Cell Proliferation

Previous work in this Laboratory utilized original methods for the electrophoretic separation and analysis of histone proteins to study the regulation of the synthesis of these proteins as a function of cell proliferation state. Although the synthesis of the major nucleosomal histones exhibited the expected regulation in phase with DNA synthesis, this was not the case for certain variant histone species, notably H2A.Z, a species that had been characterized in this Laboratory. This variant species, which replaces histone H2A in a fraction of the nucleosomes, was found to be synthesized at a slow rate even in the

absence of DNA synthesis. The basal histone syntheses that occur in the absence of DNA synthesis in GO cells relative to Gl cells were distinguishable on the basis of the relative synthesis rates of the different histones. The effects of some anti-neoplastic drugs, including DNA and protein synthesis inhibitors and DNA crosslinkers, on the pattern of histone variant synthesis was studied. A model was proposed to explain observed interactions between protein and DNA synthesis inhibitors and histone synthesis pattern.

A major component of this model involves changes in cytoplasmic histone pool sizes. However there was no reported adequate method to measure histone pool sizes. A new method was successfully developed to carry out these measurements with the required sensitivity. Utilizing this method, the state of newly synthesized histone species in the cytoplasm was observed. The possibility of contamination by histones from the nucleus was tested and was found not to be a problem.

This now opens the way to answering some key questions about the mechanisms which regulate histone synthesis. Another aspect of this problem that we are investigating concerns changes in the level of cytoplasmic histone mRNA which may be related to histone pools in a feed-back regulation loop.

In addition to the nucleosomal histones, the synthesis of histone Hl variants was studied. Again changes in the variant synthesis patterns were observed in relation to cell proliferation state and in response to DNA synthesis inhibitors.

Further insight was gained on the mechanism of the regulation of DNA and histone synthesis during S-phase. Studies were carried out of the effects of protein synthesis inhibitors applied to cells at relatively low doses such that protein synthesis was reduced by only 50%. Although DNA and histone syntheses were initially inhibited proportionately, these syntheses then recovered despite the continued inhibition of overall protein synthesis. This was found to be brought about by a compensatory elevation of histone m-RNA. A mechanism thus exists which tends to preserve the duration of S-phase when Gl is lengthened because of nutritional or metabolic deficiencies. The mechanism will be studied studied further by using other means to lengthen Gl phase. The relevance to cancer chemotherapy is that this metabolic regulatory mechanism may be defective in some neoplastic cell lines and could lead to a strategem by which such cells could be selectively killed.

In order to determine the histone variant regulatory mechanism at the gene level, we are attempting to isolate the human H2A.Z gene. Polynucleotide probes were obtained on the basis of 2 different regions of the known amino acid sequence of the protein. The probes were used as primers on mRNA derived from cells synthesizing H2A.Z. Radioactive cDNA's were then isolated and used to select clones from a human-lambda genomic library. Several clones were selected which hybridize equally with the 2 probes, and these clones are now being studied to determine whether any of them code for H2A.Z

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06140-09 LMPH

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Regulation of Histone Biosynthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Bonner	Head, Chromosome Structure and Function Section	LMPH NCI
Others:	Roy S. Wu	Cancer Expert	LMPH NCI
	Maurizio D'Incalci	Visiting Associate	LMPH NCI
	Henryk Panusz	Visiting Scientist	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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	1.9	0.4

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.)*

Our objective is to elucidate the mechanisms regulating histone biosynthesis in G1, S and G0.

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 G1 and the quiescent state (also termed extended G1 or G0). The qualitative pattern of histone synthesis differs between S-phase, G1 and quiescent cells, a finding which shows that the synthesis in G1 or quiescent cells is not due to contamination by S-phase cells. The histone synthesis in both G1 and quiescent cells is not linked to DNA synthesis. Histones synthesized in quiescent cells are stable and seem to be incorporated into chromatin. The results suggest that the quiescent state is not an extended G1 phase but a discrete state. This year, we published a comprehensive model concerning the regulation of histone and DNA synthesis. The cytoplasmic histone pool is a crucial factor in this model and methods for analyzing the histone pool are being worked out.

There is some evidence in the literature which suggests that there may be significant differences in the regulation of chromatin biosynthesis between normal and tumorigenic cells. Understanding these differences may lead to the development of protocols to protect normal cells or make tumorgenic cells more vulnerable to various treatments.

Project Description

Introduction:

We have found that when dividing cells cease division and become quiescent, they continue to synthesize histones at a reduced but significant rate. The pattern of synthesis of variant histones 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.

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 G2 and G1 (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 G1, but is a discrete state or cycle.

Objectives:

1) The development of greater understanding of the molecular mechanisms regulating chromatin biosynthesis and metabolism during the cellular states of proliferation and nonproliferation.

2) The characterization of chromatin or cellular components that may be involved in these regulatory mechanisms.

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 and nuclei in viable non dividing states using modified and defined media.

(5) Isolation and analysis of mRNA from different parts of the cell cycle or from quiescent cells. Cell free translation of mRNA.

Major Findings and Accomplishments:

1. Effects of Inhibitors of DNA and Protein Synthesis on G1, 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. We have continued these studies with a variety of inhibitors of DNA synthesis. Those agents which inhibit DNA synthesis only, give the same results as hydroxyurea. However other agents which also inhibit protein syntheses do not selectively inhibit the synthesis of particular histone variants but change the histone synthesis pattern in other ways. The crosslinking antineoplastic agents are in this class. It has previously been reported that inhibition of protein synthesis blocks the specific effect of DNA synthesis inhibitors on histone synthesis. We have recently published a model that offers an explanation for these interactions.

2. Development of Methodology to Study Histone Pools

The amount of histone in the cytoplasm is thought to be a central control element in the regulation of histone and DNA synthesis. However, the histone pool has not been studied in any systematic way because of several technical difficulties. These include the small size of the pool, the problems of purifying histone proteins from the cytoplasm, and the problem of distinguishing cytoplasmic histone from contaminating chromatin histone. Those few studies of histone pools have examined the labeling kinetics of some of the histone proteins rather than their mass.

We have adapted our methodology for the analysis of histone variants in chromatin to the analysis of histones in the cytoplasm and have overcome most of these problems. Newly synthesized H4 in the cytoplasm is doubly modified, by an acetate and a phosphate. When cytoplasm is prepared by gentle lysis of cells with a nonionic detergent, then extracted with HCl and the extract freeze dried for electrophoresis on AUT-AUC gels, the pattern of cytoplasmic histone shows doubly modifed H4 as well as the absence of ubiquitin adducts of the H2A's. Thus cytoplasmic histone can be analyzed with little or no contamination from nuclear histone.

We plan to use this newly developed method to study several aspects of the histone pool. The first set of questions concern the relationship of the pool histone to chromatin histone during normal S-phase. Do histones flux in one direction only from polyribosomes, through the pool to chromatin, or do histones flux out of chromatin into the pool? Are histones in the pool degraded? The second set of questions concern how the histones in the pool react to the inhibition of DNA and/or protein synthesis. Do the levels of histones in the pool change in such a way as to be consistent with autoregulation. The third set of questions concern the level and flux of histone in G1 and GO as well as in S, and the transition between these states.

3. In Vitro Assay for Aspects of Histone mRNA Regulation

Our specific aim on this project is to develop an assay for the selective degradation of S-phase histone mRNA. There are no reports in the literature of such an assay. When whole cells are treated with hydroxyurea to inhibit DNA synthesis, histone mRNA disappears with a half-life of about 10 minutes. Other mRNA's, such as actin mRNA, are not affected. Using lysolecithin to make cells leaky, we can prepare an extract which continues translation. By using dot blotting, we can easily measure mRNA levels in the extracts and measure rates of degradation.

The specific degradation of histone mRNA is presumably catalyzed by a nuclease, the activity of which can be rapidly modulated by a signal generated by the rate of DNA synthesis. This signal might be the level of the cytoplasmic histone pool. The significance of such a study is that if such an in vitro assay could be developed, this regulatory mechanism could be separated into its component parts.

4. Histone H1 Biosynthesis in Various Cell States

The pattern of H1 variant synthesis is very different between growing and quiescent cells. The synthesis of the variants H1.1 and H1.2 was negligible in quiescent IMR-90, but significant when these cells were proliferating. In resting lymphocytes only 3 variants appear to be synthesized, namely H1.3, H1.4 and H1.5, but after 50 hours of stimulation with PHA another subtype, likely to be H1.2 is synthesized in significant amounts. In IMR-90 cells treated with hydroxyurea (HU) and 10 μM cytosine arabinoside (Ara-C), the H1 synthesis pattern was found to be similar to that observed in quiescent cells.

We conducted some other studies to evaluate whether anticancer agents which arrest cells in premitotic phase interfere with H1 phosphorylation. We investigated this point using two semisynthetic epipodophyllotoxin derivatives, VP16 and VM26. Neither of them appear to inhibit the phosphorylation of H1 as assessed by ^{33}P-incorporation. The results of this study are being prepared for publication.

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 in normal and neoplastic cells.

Proposed Course:

1. To characterize the histone pool and to test the predictions of the published model as to the behavior of the pool.

2. To develop methodology to study the selective sensitivity of histone mRNA.

Publications:

1. Wu, R.S., West, M.H.P., and Bonner, W.M.: Histone protein synthesis in human and other mammalian cells. In Stein, G. and Stein, J. (Eds.): Histone Genes. New York, John Wiley & Sons, 1984, pp. 457-483.

2. Wu, R.S., and Bonner, W.M.: Pattern of histone variant synthesis and implications for gene regulation. In Kumar, A. (Ed.): Eukaroytic Gene Expression. New York, Plenum, 1984, pp. 37-67.

3. Seiler-Tuyns, A., Pantazis, P., Bonner, W., Hamer, D., and Kumar, A.: A mouse histone H4 gene carried by an SV40 vector is accurately expressed in infected monkey cells. DNA 3: 215-222, 1984

4. Pantazis, P., West, M.H.P., and Bonner, W.M.: Phosphorylation of histones in cells treated with hypertonic and acidic media. Mol. Cell. Biol. 4: 1186-1188, 1984.

5. Pataryas, T.A., Sekeri-Pataryas, K.T., Bonner, W.M., and Aleporou-Marinou, V.: Histone variants of the insect Plodia interpunctella during meta morphosis. Comp. Biochem. Physiol. 77B: 749-753, 1984.

6. West, M.H.P., Wu, R.S., and Bonner, W.M.: Polyacrylamide gel electrophoresis of small peptides. Electrophoresis 5: 133-138, 1984.

7. Mueller, R.D., Yasuda, H., Bonner, W.M., and Bradbury, E.M.: Identification of ubiquitinated H2A and H2B in Physarum Polycephalum: Disappearance of these proteins at metaphase and reapperance at anaphase. J. Biol. Chem. 260: 5147-5153, 1985.

8. Pantazis, P., and Bonner, W.M.: Specific alterations in the pattern of histone 3 synthesis during conversion of human leukemic cells to terminally differentiated cells in culture. Differentiation 28: 186-190, 1984.

9. West, M.H.P., Pantazis, P., and Bonner, W.M.: Studies on nuclease digestion of chromatin phosphorylated in vivo. J. Biol. Chem. 260: 4558-4560, 1985.

10. Sariban, E., Wu, R.S., Erickson, L.C., and Bonner, W.M.: Interrelationship of protein and DNA synthesis during replication in mammalian cells. Mol. Cell Biol., in press.

11. Wu, R.S., Panusz, H., Hatch, C.L., and Bonner, W.M.: Histones. CRC Crit. Rev., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06150-04 LMPH

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Protein-associated DNA Strand Breaks as Indicator of Topoisomerase II Inhibition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	Jacqueline Wang-Peng	Sr. Investigator	MB NCI
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	Donna Kerrigan	Chemist	LMPH NCI
	Ronald E. Schwartz	Biologist	LMPH NCI
	Kurt W. Kohn	Lab Chief	LMPH NCI

COOPERATING UNITS *(if any)*

Merck Research Institute, West Point, PA (Dr. M. O. Bradley); LCP, DTP, NCI; MCPB, COP, NCI; Institut Gustave Roussy (Dr. A. Jacquemin-Sablon) Villejuif, France

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

DNA Damage and Repair Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2	2	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 protein-associated DNA single and double-strand breaks induced in mammalian cells by DNA intercalating agents and epipodophyllotoxins have been shown to represent an effect on topoisomerase II. The aim of this project is to investigate the relationship between the drug-induced protein-associated strand breaks and the physiological state and pharmacological sensitivity of cells. A correlation was found in V79 cells between protein-associated double-strand breaks and sister-chromatid exchange, mutation and cytotoxocity. A multi-drug resistant line of Chinese hamster cells exhibited reduced strand break responses to the intercalators, amsacrine and ellipticine, and to the epipodophyllotoxin, etoposide; the uptake of these drugs into the resistant cells was not altered. Protein-associated strand breakage in response to amsacrine and etoposide was found to be dependent upon the proliferation state of NIH 3T3 cells; quiescent cells showed low sensitivity to this response, as well as to cell killing; when cells were stimulated to proliferate, the responses increased during the onset of DNA synthesis. Further work aims to elucidate the changes in topoisomerase(s) in resistant cells.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

1. Determine the role of topoisomerase II in the production of sister chromatid exchanges, mutations and cell killing in mammalian cells.

2. Determine the cytotoxic role of topoisomerase II trapping by intercalators and epipodophyllotoxins by comparing sensitive and resistant cell lines.

3. Study the mechanism of cell resistance to topoisomerase II inhibitors.

4. Determine the cell cycle-dependence of the protein-associated DNA strand breaks and cytotoxicity produced by intercalators and epipodophyllotoxins.

Methods:

- Standard cell culture and synchronization methods.

- Measurement of protein-associated DNA strand breaks by alkaline elution.

- Measurement of sister chromatid exchanges, mutations and cytotoxicity by standard procedures.

- Topoisomerase II (and nuclear protein) isolation by gel filtration, DNA affinity chromatography and glycerol gradient centrifugation.

Major Findings:

1. Correlations Between Intercalator-induced DNA Strand Breaks and Sister Chromatid Exchanges, Mutations and Cytotoxicity in Chinese Hamster Cells

Intercalator-induced DNA strand breaks in mammalian cells represent topoisomerase II-DNA complexes trapped by intercalators. These complexes are detected as protein-associated DNA single-strand breaks (SSB) and DNA double-strand breaks (DSB) by filter elution. Using Chinese hamster lung fibroblasts (V79 cells) that were treated for 30 min with various concentrations of 4'-(9-acridinylamino) methanesulfon-m-anisidide (amsacrine) or 5-imino-daunorubicin (5-ID), we measured DNA strand breaks (SSB & DSB), sister chromatid exchanges (SCEs), mutations at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus and cell killing. Further, we correlated DNA strand breakage with the three other parameters. Both drugs induced SCEs, mutations and cell killing at concentrations which also produced reversible DNA strand breaks. While the quantity of DSB correlated with SCEs, mutations and cytotoxicity for both drugs, we found more SCEs, mutations and cytotoxicity per SSB in cells treated with 5-ID than in those treated with amsacrine. These data show that the DSB (but not the SSB) induced by amsacrine and 5-ID at DNA topoisomerase II binding sites correlated closely with SCEs, mutations and cell killing, and could therefore be responsible for their production. The mechanism by which intercalators induce sister chromatid exchanges (SCEs) is not known. We propose that these drugs stimulate a subunit exchange of topoisomerase II molecules within cleavable complexes at DNA replication forks. The subunit exchange would lead to a SCE after an additional round of DNA replication. Details of this model have been discussed in our publication (Pommier et al., Cancer Res., 1985, in press). We also propose that the

trapping of topoisomerase cleavable complexes (formation of DNA double-strand breaks) could lead to frame shift mutations.

2. Role of Topoisomerase II Inhibition (Formation of Cleavable Complexes) by Intercalators and Epipodophyllotoxins in the Cell Killing (Antitumor) Effect of these Drugs

Our approach has been to use cells resistant to topoisomerase II inhibitors. A Chinese hamster cell line (DC3F/9-OH) had been made resistant to 9-hydroxy-ellipticine by Dr. A. Jacquemin-Sablon (Institut Gustave Roussy, Villejuif, France). We selected these cells because 1) they had been made resistant to a drug which is a potential topoisomerase II inhibitor; 2) the parent cell line (DC3F) could be used as a control; and 3) the resistance to ellipticines was not due to a reduced cellular uptake of the drug. Resistant cells (DC3F/9-OH) were first tested for their cross-resistance to ellipticinium, amsacrine and etoposide (VP-16). DC3F/9-OH cells appeared to be highly resistant not only to the two intercalators (amsacrine and ellipticinium) but also to etoposide which does not bind DNA. Resistance to amsacrine was not due to a modified uptake of the drug. Additional drug transport studies showed that resistance to adriamycin was, on the other hand, associated with a reduced uptake of the drug.

We then measured the formation of protein-associated DNA strand breaks in both cell lines. Resistant DC3F/9-OH cells produced markedly less protein-associated DNA single- and double-strand breaks than sensitive DC3F cells after a 30 min exposure to amsacrine, ellipticinium or etoposide. Similar results were found by treating isolated nuclei from each cell line.

These results show that resistance to intercalators and etoposide can occur without drug uptake change in cells which, however, may have plasma membrane modifications (reduced uptake of adriamycin). In addition, the results suggest that 1) resistance to amsacrine, ellipticinium and etoposide could be due to the reduced trapping of topoisomerase II within cleavable complexes, and 2) topoisomerase II modifications are present in resistant (DC3F/9-OH) relative to sensitive (DC3F) cells.

This last point has been further investigated. DNA from DC3F/9-OH (resistant) cells has been isolated and transfected to mouse NIH 3T3 cells. 7 resistant transfected cell lines have been obtained. The integration of Chinese hamster (DC3F/9-OH) DNA into mouse cells (3T3) was confirmed by dot-blot hybridization. The resistant transfected cells (NAD) appeared to be as resistant to amsacrine as were the donor resistant cells. NAD cell nuclei, produced also much less protein-associated DNA breaks than did 3T3 cell nuclei NAD cells had also lost their reduced adriamycin uptake. These results suggest that resistance to intercalators and etoposide in DC3F/9-OH cells is due to the presence of a factor that reduces the ability of topoisomerase II to be trapped by intercalators and etoposide.

Additional data support this hypothesis. Purified topoisomerase II from DC3F/9-OH cells was as sensitive to amsacrine as that from DC3F or L1210 cells. Nuclear extracts from DC3F/9-OH cells, however, did not produce amsacrine-dependent topoisomerase II cleavable complexes. It appears therefore that a modulating factor that reduced the effect of amsacrine maybe present

in the nuclear extract from resistant cells. The nature of this modulating factor is yet unknown. The fact that crude nuclear extracts from DC3F/9-OH cells and from resistant transfected cells (NAD) had an unusually high level of DNA linking activity suggests that this DNA linking activity might play a role as a modulating factor.

3. Protein-associated DNA Breaks in Relation to Cell Cycle

Previous experiments (Minford et al., Cancer Res., 1984) suggested that intercalator-induced protein-associated DNA breaks may be increased in replicating cells.

Mouse 3T3 cells stop proliferation when they reach a certain density on the plate. The cells can be initiated to proliferate by replating at a lower density and feeding with fresh medium containing calf serum. We found that amsacrine-dependent DNA breakage in proliferating cells was much greater than in arrested cells. We also studied the DNA breakage as a function of time following replating of the cells and found peaks of DNA synthesis at 5-6 h and about 10 h after replating the cells into fresh medium. Assays were done by taking cells at various times, and incubating them for 30 min with 10 μM amsacrine. Filter elution assays for DNA double-strand breaks were then carried out, and showed peaks of DNA breakage occurring at about the times of the peaks of [^{3}H]-thymidine incorporation.

Proposed Course:

1. Study the formation of sister chromatid exchanges (and mutations) in DC3F/9-OH resistant cells in order to further correlate the formation of sister chromatid exchanges to that of protein-associated DNA breaks and the possible involvement of topoisomerase II in sister chromatid exchange formation and cell killing (antitumor activity).

2. Isolate purified topoisomerase II from sensitive (DC3F) and resistant (DC3F/9-OH) cells and compare their cleavage pattern in the absence and presence of amsacrine, ellipticinium and etoposide. This could be done in sequencing gels.

3. Re-transfect the DNA from the first generation of transfected cells (NAD) into NIH 3T3 cells in order to isolate the gene(s) involved in resistance to topoisomerase II inhibitors and the modulating activity of topoisomerase II, which seem to be associated with drug resistance.

4. Further study the cell cycle-dependence of amsacrine-induced protein-associated DNA breaks by performing experiments in isolated nuclei. This procedure should limit drug uptake differences during cell cycle and allow a better analysis of the relationship between topoisomerase II-induced DNA breaks and DNA synthesis. We plan also to study the cell cycle-dependence of amsacrine cytotoxicity and etoposide-induced protein associated DNA breaks and cyto toxicity.

Publications:

1. Minford, J., Kerrigan, D., Nichols, M., Shackney, S., and Zwelling, L.A.:

Enhancement of the DNA breakage and cytotoxic effects of intercalating agents by treatment with sublethal doses of 1-β-D-Arabinofuranosylcytosine or hydroxyurea in L1210 cells. Cancer Res. 44: 5583-5593, 1984.

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3. Pommier, Y., Jacquemin-Sablon, A., Schwartz, R.E., and Kohn, K.W.: Alterations of DNA topoisomerases in Chinese hamster cells resistant to DNA intercalators and VP-16. Proc. Am. Cancer Res. 26: 344, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06160-02 LMPH

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Mechanism of Action of DNA Crosslinking Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.4	2.4	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.)*

Chloroethylnitrosoureas (ClEtNUs) kill susceptible human tumor cells by chloroethylation of guanine-O6 positions in DNA, leading to the production of interstrand crosslinks. Chloroethylating agents potentially superior to ClEtNUs are being studied. 2-chloroethyl(methylsulfonyl)methanesulfonate ("ClEtSoSo") (NSC 338947) was found to have the desirable feature of lacking a prominent chemical side-reaction, hydroxyethylation of DNA bases, produced by the ClEtNUs. In reactions with purified DNA, ClEtSoSo produced only chloroethyl products whereas a ClEtNU produced more hydroxyethylation than chloroethyl products. Despite the lack of hydroxyethylation reaction, ClEtSoSo is at least as effective as any ClEtNU against experimental tumors.

Another chloroethylating agent, mitozolomide, produced a greater diversity of products than did the ClEtNU; this was considered to be an undesirable feature of this drug.

The susceptibility of certain human tumor cell lines to ClEtNUs depends on a deficiency of guanine-O6-alkyltransferase. New methods are being developed for isolating this enzyme from human liver and for assaying the enzyme activity in tumor tissues.

Studies are in progress to test for relationships between clinical response of malignant glioma patients and DNA crosslinking responses of cells from these patients' tumors to aziridinylbenzoquinone.

Project Description

Objectives:

1. Define the mechanism of action of new anticancer drugs that kill susceptible human tumor cells by chloroethylation of DNA guanine-O6 positions; find compounds that produce predominantly this reaction with a minimum of side-reactions.

2. Develop improved methods of isolation and assay of guanine-O6-alkyltransferase, the key enzyme that determines the susceptibility of human tumor cells to chloroethylating agents; investigate the properties of this enzyme and clone genes in order to determine why the enzyme is deficient in certain cells.

3. Test for correlations between clinical responses to DNA crosslinking drugs and crosslinking responses in cell cultures derived from the tumors.

Methods:

1. Reaction of radioactive drugs with DNA.

2. Separation of reaction products by high pressure liquid chromatography (HPLC).

3. Organic synthesis of predicted purine adducts.

4. Mass spectrometry identification of purine adducts.

5. Alkaline elution methods developed in this Laboratory for the determination of DNA lesions in mammalian cells.

6. Chromatographic methods of enzyme isolation.

7. Restriction enzyme assay of synthetic oligonucleotides containing an abnormal base.

8. Gene transfer by DNA transfection and detection of transfered DNA by molecular hybridization.

Major Findings:

2-Chloroethyl(methanesulfonyl)methanesulfonate ('ClEtSoSo') (NSC 338947)

This compound (colloquially 'ClEtSoSo') is being considered for clinical development by NCI as a new type of alkylating agent. We had found ClEtSoSo to produce DNA interstrand crosslinking and DNA-protein crosslinking in Mer^- (guanine-O6-alkyltransferase deficient) human tumor cell lines, the effects being indistinguishable from those produced by 2-chloroethylnitrosoureas (ClEtNUs) of the non-carbamoylating type. ClEtSoSo selectively killed Mer^- relative to Mer^+ human tumor cells, as did ClEtNUs, and the degree of selectivity was even greater than that produced by any ClEtNU tested.

The major question addressed during the current year was whether, as was expected on chemical grounds, ClEtSoSo would produce a more restricted range of of reaction products with DNA than do the ClEtNUs. ClEtNUs were known to produce hydroxyethyl adducts of DNA bases as major products, and it appeared unlikely that these reactions would contribute to antitumor activity.

Purified DNA was therefore reacted with [^{14}C-ethyl]-labelled ClEtSoSo and ClEtNU (the non-carbamoylating cis-2-OH-CCNU derivative was used). The DNA was hydrolysed and the reaction products were separated by HPLC. Whereas cis-2-OH-CCNU yielded 5 HPLC peaks, ClEtSoSo produced only 3 peaks. Two of the peaks were identified by chemical synthesis and mass spectrometry: N7-chloroethylguanine, and N7-hydroxyethylguanine. Whereas cis-2-OH-CCNU produced all both of these products (and in fact more N7-hydroxyethyl than N7-chloroethylguanine), ClEtSoSo by contrast produced N7-chloroethyl-guanine and no detectable hydroxyethyl product.

The results support our premise that ClEtSoSo is free of certain side reactions, namely hydroxyethylation reactions, which are major reactions of all ClEtNUs. The fact that ClEtSoSo at least equalled any ClEtNU in its effectiveness against a broad spectrum murine tumor systems in special NCI tests gives additional support to the proposition that hydroxyethylation reactions are unnecessary for antitumor activity.

Mitozolomide (M&B 39565)

This compound was developed in England as a possible pro-drug for chloroethyl triazenoimidazolecarboxamide (ClEtTIC) and is undergoing clinical trial in Europe. We found that mitozolomide and ClEtTIC produce interstrand crosslinks and DNA-protein crosslinks and exhibit selective actions on Mer$^-$ cells in a manner similar to the ClEtNUs. ^{14}C-chloroethyl-labeled mitozolomide was studied along with ClEtSoSo and ClEtNU in the studies described above. Mitozolomide generated DNA reaction products which on HPLC separation yielded 9 peaks, an even greater number than was produced by the ClEtNU. Among these peaks were all 3 hydroxyethyl and chloroethylguanine species mentioned above, with the N7-hydroxyethyl predominating over the N7-chloroethylguanine.

These results indicate that mitozolomide generates an even greater diversity of chemical reactions than do (non-carbamoylating) ClEtNUs. From the point of view of the strategy to reduce reaction diversity and to develop compounds which produce only the necessary reactions, the clinical development of mitozo-lomide would not be supported.

Guanine-O6-alkyltransferase (GO6AT)

A deficiency of GO6AT activity makes some human tumor cells (Mer$^-$ phenotype) susceptible to killing by chloroethylating agents. When GO6AT activity is normal (Mer$^+$ phenotype), chloroethyl adducts are rapidly removed from guanine-O6 positions. When the enzyme is deficient (Mer$^-$ cells), the removal of these adducts is reduced or delayed, and the adducts react further to produce potentially lethal interstrand crosslinks. These conclusions, which are now generally accepted on the basis of work from this and other laboratories, show the importance of understanding the properties of GO6AT and the origin of the deficiency in Mer$^-$ cells.

A new procedure was developed for purification of GO6AT from human liver. The steps are: (1) preparation of extract by Waring blender; (2) ammonium sulfate precipitation and storage; (3) Affigel Blue chromatography; (4) mercury-agarose chromatography; (5) single-strand DNA agarose chromatography; (6) phenylagarose chromatography. The aim is to purify enough human enzyme for antibody production and amino acid sequencing.

As an approach to cloning of the gene, DNA transfer of GO6AT from human Mer^+ to Mer^- cells was attempted. Co-transfer of the pSVneo gene was used to aid the selection. A clone was isolated which contained GO6AT activity associated with a 22 kilodalton protein, in addition to pSV2neo sequences. Encouraged by this result, transfer was attempted into CHO cells so that the transfer of human DNA in the hamster cells could be monitored. The transfer of GO6AT activity into CHO cells however was unsuccessful.

The development of a simple and sensitive assay for GO6AT was undertaken. A deoxypolynucleotide was obtained containing O6-methylguanine at a defined position in the sequence. The sequence was designed so that the O6-methylguanine is at a restriction site which is blocked by the presence of this altered base. The assay for GO6AT activity depends on the removal of the methyl group from the O6 position which would then allow restriction endonuclease cutting at this site.

Test of Correlation Between DNA Crosslinking and Clinical Response of Malignant Gliomas

In a collaborative study with the Surgical Neurology Branch, NINCDS, we are testing for a possible correlation between clinical response to DNA crosslinking drugs and the crosslinking response of cell lines derived from the tumors. The Surgical Neurology Branch routinely establishes cultures from most of their patients operated for malignant gliomas, and the resulting cell strains are stored frozen. In the current study, the clinicians select from their records patients who they felt responded to aziridinylbenzoquinone (AZQ) and patients who failed to respond. They prepare the corresponding cell cultures from frozen stocks and forward them to us as coded samples. In the first set of cultures, 2 cell strains from responders showed greater interstrand crosslinking responses in vitro than did 2 cell strains from non-responders.

Significance:

1. It was shown that 2 major classes of chemical side-reactions can be eliminated without impairment of the experimental antitumor activity of an extraordinarily effective class of drugs. Elimination of unnecessary side-reactions may improve the therapeutic index of these drugs.

2. The studies of human guanine-O6-alkyltransferase (GO6AT) aim to provide an assay by which tumor samples can be tested for potential sensitivity to treatment with chloroethylating drugs. In addition, an understanding of the origin of the GO6AT deficit in some human tumor cells could lead to improved strategies for selective killing of potentially susceptible tumors.

3. A demonstrated positive correlation between DNA interstrand crosslinking and clinical response of individual tumors to particular drugs would be a key

step in efforts to develop more potent and specific crosslinking agents. It could also lead to predictive drug sensitivity tests.

Proposed Course:

1. Develop assays for guanine-O6-alkyltransferase (GO6AT) in tumor tissues. An immunochemical assay for GO6AT protein and a restriction enzyme assay for GO6AT activity will be developed.

2. Study possible correlations between GO6AT activity, DNA crosslinking and the sensitivities of various human tumor cell types and of patients bearing the tumors to DNA crosslinking drugs.

3. Study possible synergism between DNA crosslinking agents and epipodophyllotoxins.

Publications:

1. Szmigiero, L., Erickson, L.C., Ewig, R.A.G., and Kohn, K.W.: DNA strand scission and cross-linking by diaziridinylbenzoquinone (diaziquone) in human cells and relation to cell killing. Cancer Res. 44: 4447-4452, 1984.

2. Szmigiero, L., and Kohn, K. W.: Mechanisms of DNA strand breakage and interstrand cross-linking by diaziridinylbenzoquine (diaziquone) in isolated nuclei from human cells. Cancer Res. 44: 453-4457, 1984.

3. Sariban, E., Erickson, L. C., and Kohn, K. W.: Effects of carbamoylation on cell survival and DNA repair in normal humane embryo cells (IMR-90) treated with various 1-(2-chloroethyl)-1-nitrosoureas. Cancer Res. 44: 1352-1357, 1984.

4. Yarosh, D.B.: The role of O^6-methylguanine-DNA methyltransferase in cell survival, mutagenesis and carcinogenesis. Mutat. Res. 145: 1-16, 1985.

5. Zlotogorski, C., and Erickson, L.C.: Pretreatment of human colon tumor cells with DNA methylating agents inhibits their ability to repair chloroethyl monoadducts. Carcinogenesis 5: 83-87, 1984.

6. Yarosh, D.B., Rice, M., Day, R. S, III, Foote, R.S., and Mitra, S.: O^6-methylguanine-DNA methyltransferase in human cells. Mutat. Res. 131: 27-36, 1984.

7. Yarosh, D. B., Ziolkowski, C., and Day, R.S., III: Conversion of Human Cells to Carcinogen Resistance by DNA Transfection. In Bishop, J.M., Graves, M., and Rowley, J. (Eds.): Genes and Cancer. New York, Liss, in press.

PERIOD COVERED
October 1, 1984 to September 30, 1985

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Topoisomerase II as Target of Action of Anticancer Drugs

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
6.0	5.0	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 effects of DNA intercalating agents and epipodophyllotoxins are being studied upon purified mammalian topoisomerase II. DNA intercalators and epipodophyllotoxins inhibit DNA topoisomerase II while trapping the enzyme within topoisomerase II-DNA DNA cleavable complexes. The cleavable complexes consist of two enzyme subunits which are covalently bound to the 5'-termini of the DNA break. The DNA breaks can be detected only after protein denaturation by sodium dodecyl sulfate and proteinase K digestion. The breaks also reverse upon salt addition in the absence of added nucleotide. The cleavable complexes trapped by intercalators and epipodophyllotoxins are analogous to intermediates in the DNA breaking-rejoining reaction of topoisomerase II. It is by this reaction that the enzyme carries out DNA strand passage and DNA topoisomerization reaction. Another mode of interaction between topoisomerase II-DNA complexes and intercalator is the forced reversal or unlocking of cleavable complexes. This effect has been observed with high concentrations of 2-methyl-9-hydroxyellipticinium and with the bisintercalator, ditercalinium. The unlocking of cleavable complexes seems to be related to DNA binding and may not be accompanied by enzyme inhibition.

The localization of intercalator-induced topoisomerase II action in the nuclear genome was investigated. It was found that certain repeated sequences are enriched at a favored distance from sites of intercalator-induced topoisomerase II-DNA linkage. The results have implications regarding the localization of topoisomerase II action in the loop structure of nuclear chromatin.

Objectives:

1. Determine the role of topoisomerase II in the formation of protein-associated DNA breaks by intercalators and epipodophyllotoxins in mammalian cells.

2. Study the modes of interaction between DNA intercalators or epipodophyllotoxins and topoisomerase II-DNA complexes.

3. Determine the mechanisms(s) by which topoisomerase II recognizes potential DNA cleavage sites within isolated DNA.

4. Investigate the localization of topoisomerase II action in the nuclear genome.

Methods:

1. Purification of topoisomerase II and I from mammalian cells by 1) Gel filtration of 0.35 M salt extract from isolated nuclei 2) DNA-cellulose chromatography 3) Glycerol gradient centrifugation. SDS-PAGE electrophoresis is used to test enzyme purity.

2. Filter binding assay to detect the formation of covalent complexes between radioactively labeled DNA ([^{3}H]-SV40) and topoisomerases II and I.

3. Electrophoresis of radioactively [^{32}P]-labeled DNA (pBR 322, SV-40) in in agarose or in polyacrylamide-urea (sequencing methods) gels to detect the sequence specificity of the topoisomerase II-induced DNA double- and single-strand breaks respectively.

4. Controlled DNA scission, electrophoresis, blotting and DNA hybridization.

Major Findings:

Effects of the DNA Intercalators, Amsacrine (m-AMSA) and Ellipticinium (2-methyl-9-hydroxyellipticinium) Upon L1210 DNA Topoisomerase II

Previous studies have indicated that the protein-associated DNA strand breaks detected in mammalian cells treated with intercalating drugs are produced by topoisomerase II and that the enzyme becomes covalently bound to DNA at the termini of the breaks.

Using isolated nuclei preparations we have found an unexpected difference between amsacrine and ellipticinium. Amsacrine produced DNA double-strand breaks at all concentrations, and the yield of breaks tended towards saturation, leading to approximately one DNA double-strand break per 5×10^{5} base pairs. Ellipticinium, on the other hand, produced more complicated effects. At concentrations below 10 μM, DNA breaks were produced whereas at higher concentrations the breaks were not formed. The lack of break formation at high ellipticinium concentrations was due to an active inhibition of the DNA cleavage reaction because at these same concentations, the amsacrine-induced breaks were also inhibited by ellipticinium.

By performing experiments where we mixed amsacrine and ellipticinium, it appeared that the production of breaks by low ellipticinium concentrations and the inhibition of the breaks by high ellipticinium concentrations, resulted from two opposite but competitive modes of interactions upon the same nuclear target enzymes.

Similar types of experiments were performed using topoisomerase II purified from L1210 nuclei. Amsacrine produced both DNA single- and double-strand breaks in the presence of topoisomerase II.

These breaks were inferred to be associated with enzyme covalently bound to the 5'-termini of the DNA breaks. The enzyme-associated DNA breaks were produced by amsacrine at concentrations ranging from 1 to 20 μM. At the same time that the breaks were formed, the DNA topoisomerization reactions of topoisomerase II (ATP-dependent DNA relaxation and catenation) were inhibited. As observed in the nucleus preparations, ellipticinium had more complicated effects upon topoisomerase II. At low concentrations (below 2 μM), ellipticinium produced enzyme-associated DNA breaks and enzyme inhibition whereas at high concentrations (above 5 μM), it inhibited the protein-associated DNA breaks formed by the topoisomerase II alone or in the presence of amsacrine. This "unlocking" effect of high ellipticinium concentrations did not seem to be associated with enzyme inhibition.

These results show that two modes of interactions exist between intercalators and topoisomerase II-DNA complexes: 1) trapping of enzyme-DNA complexes leading to the formation of cleavable complexes and enzyme inhibition; 2) unlocking (forced reversal) of cleavable complexes leading to the inhibition of topoisomerase II-induced DNA breaks in the absence of inhibition of enzyme activity. The similarities between the results obtained with purified topoisomerase II and those obtained in isolated nuclei, further supports the inference that the protein-associated DNA breaks produced by antitumor intercalating drugs are mediated by topoisomerase II and associated with enzyme inhibition.

Effects of the Bisintercalator, Ditercalinium Upon L1210 DNA Topoisomerase II

Ditercalinium consists of 2 intercalative rings (7H pyridocarbazole) linked by 2-$N^{+}(CH_2)_2$-1-piperidyl rigid chain. The rigidity of the linking chain prevents the stacking of the 2 intercalative rings and the folding of the molecule.

Treatment of L1210 cells with ditercalinium at concentrations lower than 2 μM for 24 hr did not produce any detectable DNA damage. At concentrations above 2 μM the DNA was extensively degraded and an immediate cytolysis was observed. In addition, nucleoids from ditercalinium treated cells were more compact than those from untreated cells at drug concentrations where no DNA damage could be detected. This nucleoid compaction was not produced in L1210 cells that had been made resistant to the drug. These complexes results suggest that ditercalinium does not trap topoisomerase II cleavable complexes as do monointercalators. In addition, the results of the nucleoid sedimentation experiments suggests that chromatin compaction is a major effect of ditercalinium.

The effects of ditercalinium were then studied upon purified L1210 topoisomerase II. By contrast to monointercalators, ditercalinium did not stimulate the formation of topoisomerase II cleavable complexes. Ditercalinium, however, altered topoisomerase II activity in three ways.

1) Ditercalinium prevented the formation of cleavable complexes formed by the enzyme alone or in the presence of amsacrine and etoposide (VP-16). This effect was comparable to the "unlocking" effect of high elliptinium concentrations. 2) Ditercalinium inhibited the relaxation of SV-40 DNA by topoisomerase II in the presence of ATP. This inhibition seemed to be related to DNA unwinding rather than to a direct enzyme inhibition. 3) Ditercalinium stimulated DNA catenation (or aggregation) by topoisomerase II. This stimulation was maximum between 0.1 and 1 μM of ditercalinium and was ATP-dependent. Ditercalinium concentrations above 1 μM inhibited the catenation reaction.

Effects of the Epipodophyllotoxins, Etoposide (VP-16) and Teniposide (VM-26) Upon L1210 DNA Topoisomerase II

Epipodophyllotoxins have been reported to induce protein-associated DNA breaks in mammalian cells and to stimulate the formation of cleavable complexes formed by calf thymus topoisomerase II. The possible interaction of epipodophyllotoxins with topoisomerase II plus the fact that these drugs are active antitumor agents that do not bind DNA, prompted us to investigate their mechanism of action.

The production of DNA breaks and DNA-protein crosslinks by etoposide and teniposide was first studied in intact cells. Both compounds produced DNA strand breaks and DNA protein crosslinks in mouse leukemia L1210 cells, in human colon carcinoma HT-29 cells and in viral transformed human embryonic VA-13 cells. At all drug concentrations the frequency of DNA strand breaks was equal to the frequency of DNA-protein crosslinks within experimental error. In addition, no DNA breaks were detected under nondeproteinizing conditions. Similar results were obtained by treating isolated nuclei with etoposide and teniposide. It appeared therefore that the DNA strand breaks produced by etoposide and teniposide were protein-associated and probably represent topoisomerase II cleavable complexes. Two additional observations were made from the studies performed in whole cells and isolated nuclei: 1) teniposide was several times more potent than etoposide at equimolar concentrations, and 2) the two human cell lines (HT-29 and VA-13) produced approximately 3 times less protein-associated DNA strand breaks than the mouse leukemia L1210 cells. The production of protein-associated DNA strand breaks thus varies with the compound and cell line used.

The effects of epipodophyllotoxins were then studied on purified L1210 topoisomerase II. Both etoposide and teniposide stimulated the formation of cleavable complexes and inhibited the DNA relaxing activity of topoisomerase II. The formation of cleavable complexes was stimulated by ATP and by its nonhydrolyzable analog, ATP-γ-S. In agreement with the results obtained in cells, we found that teniposide was approximately 10-fold more potent than etoposide. The trapping of cleavable complexes by epipodophyllotoxins appeared to be different from that produced by amsacrine: 1) It was resistant to the

reversing effect of 0.5 M NaCl, and 2) the location of the cleavable complexes within pBR 322 were different for epipodophyllotoxins and of amsacrine.

The present data strongly suggest that the protein-associated DNA breaks produced by the epipodophyllotoxins, etoposide (VP-16) and teniposide (VM-26), are due to the trapping of topoisomerase II cleavable complexes and are associated with enzyme inhibition. In addition, the cleavable complexes trapped by epipodophyllotoxins differ in some respects from those trapped by amsacrine.

Genomic Localization of Intercalator-induced Protein-Associated Strand Breaks (PASBs)

The possibility was investigated that intercalator-induced PASBs occur selectively at certain locations in the genome. Methods were devised for the isolation of DNA sequences close to the sites of intercalator-induced protein linkage, presumably linkages between DNA strand termini and topoisomerase II. Molecular hybridization of dot-blots and of Southern blots indicated that $(TG)_n$ (n>10-20) sequences in both mouse and human DNA were enriched at a distance of about 6 kilobases from the sites of protein linkage. A similar enrichment was observed for the Alu class of repeated sequences in human cells. The results were not entirely as expected and can be interpreted in terms of the loop structure of chromatin with special functions involving topoisomerase II bound to the nuclear scaffold.

Proposed Course:

1. Map the cleavage sites of topoisomerase II using DNA sequencing techniques and [^{32}P]-labeled DNA (pBR 322 and SV-40). Various compounds will be used to stimulate the formation of cleavable complexes (amsacrine, elliptinium, epipodophyllotoxins).

2. Determine whether there is any consensus among the DNA sequences at which topoisomerase II cleaves DNA. Determine the recognition sequences of topoisomerase II.

3. Use acridine derivatives having different DNA affinities and different DNA binding specificities (GC versus AT). If the trapping of cleavable complexes is determined by drug intercalation sites, the cleavage pattern should vary from one compound to another depending on the binding sites of the drugs.

Publications:

1. Pommier, Y., Mattern, M.R., Schwartz, R., and Zwelling, L.A.: Absence of swivelling of sites of intercalator-induced protein-associated deoxyribonucleic acid strand breaks in mammalian cell nucleoids. Biochemistry 23: 2922-2927, 1984.

2. Pommier, Y., Mattern, M.R., Schwartz, R., Zwelling, L.A., and Kohn, K.W.: Changes in deoxyribonucleic acid linking number due to treatment of mammalian cells with the intercalating agents, 4'-(9-acridinylamino) methanesulfon-m-anisidide (m-AMSA). Biochemistry 23: 2927-2932, 1984.

3. Pommier, Y., Schwartz, R.E., Kohn, K.W., and Zwelling, L.A.: Formation and rejoining of deoxyribonucleic acid double-strand breaks induced in isolated cell nuclei by antineoplastic intercalating agents. Biochemistry 23: 3194-3201, 1984.

4. Pommier, Y., Zwelling, L.A., Schwartz, R. E., Mattern, M.R., and Kohn, K.W.: Absence of a requirement for long range DNA torsional strain in the the production of protein-associated DNA strand breaks in isolated mammalian cell nuclei by the DNA intercalating agent 4'-(9-acridinylamino) methanesulfon-m-aniside (m-AMSA). Biochem. Pharmacol. 33: 3909-3912, 1984.

5. Pommier, Y., Schwartz, R.E., Zwelling, L.A., and Kohn, K.W.: Effects of DNA intercalating agents on topoisomerase II-induced DNA strand cleavage in isolated mammalian cell nuclei. Biochemistry, in press.

6. Pommier, Y., Minford, J.K., Schwartz, R.E., Zwelling, L.A., and Kohn, K.W.: Effects of the DNA intercalators, 4'-(9-acridinylamino)methanesulfon-m-aniside (m-AMSA, amsacrine) and 2-methyl-9-hydroxyellipticinium (2-Me-9-OH-E+) on topoisomerase II-mediated DNA strand cleavage and strand passage. Biochemistry, in press.

7. Minford, J.K., Pommier, Y., Filipski, J., Kohn, K.W., Kerrigan, D., Mattern, M.R., Michaels, S., Schwartz, R.E., and Zwelling, L.A.: Isolation of intercalator-dependent protein-linked DNA strand cleavage activity from cell nuclei and identification as DNA topoisomerase II. Biochemistry, in press.

8. Minford, J.K., Pommier, Y., Uhlenhopp, E., Mattern, M.R., Kerrigan, D., Mattes, W., and Kohn, K.W.: Topoisomerase II activity of cells, nuclei and in vitro is a target for the epipodophyllotoxins. Biochemistry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06170-01 LMPH

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Isolation of Human Variant Histone Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Bonner	Head, Chromosome Structure and Function Section	LMPH NCI
Others:	Christopher Hatch	Staff Fellow	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

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	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.)*

Our objective is to isolate the genes for the human histone H2A family, in particular the H2A.Z gene.

A number of putative H2A.Z clones have been isolated and are being further screened.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Introduction:

The regulation of the various histone variants differs greatly with respect to the rates of protein and DNA synthesis. Isolation of the genes for the variant histones will help elucidate these regulatory mechanisms.

Objective:

To isolate human histone genes particularly the gene for H2A.Z.

Methods:

1. Recombinant DNA techniques.

2. Use of synthetic probes to prime reverse transcriptase and search genomic libraries.

Major Findings and Accomplishments:

In order to isolate the various histone genes, we have utilized the human genomic library originally established in Phil Leder's laboratory by the insertion of human placental DNA into the charon 28 lambda bacteriophage. A "complete" library of the human genome in lambda was grown with C600 E. coli bacteria and plated in 15 cm diameter plates. Each plate was replica plated in duplicate with sterile nitrocellulose filters. The filters were processed and incubated with radioactively labeled DNA probes.

Our primary effort has been towards the isolation of the genes encoding the minor variant form of H2A, H2A.Z. The isolation of non-S-phase histone genes has been difficult. This problem is most acute in the case of the gene for histone H2A.Z., since the amino acid sequence of this polypeptide has been found to be quite divergent from that of the other histone H2A sequences while it appears that the amino acid sequence of H2A.Z is conserved in a wide variety of species, the nucleotide sequences encoding this polypeptide may be more divergent between species. An H2A.Z related gene, called H2A.F, has been isolated from chicken, but cross-hybridization of this gene to homologous sequences in human, mouse, or sea urchin DNA was not found. This demonstrated that rather divergent nucleotide sequences could be coding for the same protein sequence and reinforced our belief that it would be difficult to utilize heterologous histone H2A gene probes to search for the human H2A.Z gene sequence. A more general approach not subject to these drawbacks involves the preparation of probes from known amino acid sequences. These specific probes for the detection of the desired histone mRNA's or genes can be obtained by the chemical synthesis of oligonucleotides which represent all possible codon combinations for a short run of amino acids in a given protein. H2A residues 24-28 were of particular interest to us since it has been determined that all H2A histone variants which had been sequenced in plants and animals have a common amino acid sequence from residues 21 to 29. We designed a 14-nucleotide length oligonucleotide sequence mixture that would contain sequences in this region. The probe designed contained a mixture of 14-base long synthetic oligonucleotides containg 16 possible combinations of sequence.

In order to both increase the specificity and stability of hybridization between the designed probe and the homologous sequences within the human genomic lambda library the synthetic oligonucleotide mixture was utilized as a primer for making partial cDNA copies of mRNA onto which it hybridized. In order to increase the proportion of H2A.Z mRNA relative to the other H2A mRNA's the mRNA treated with hydroxyurea for one hour prior to RNA purification from these cells. This treatment reduces the amount of major H2A variant mRNA and therefore would effectively increase the relative proportion of minor H2A variant mRNA. The oligonucleotide probe mixture was used to prime synthesis of radioactive cDNA copies of template mRNA. The ^{32}P-labeled cDNA's were then used to probe the human genomic lambda library. On the order of 1/500-1/1000 of the human-lambda clones were observed to hybridize the radioactive probes.

We decided to make a new synthetic probe mixture complementary to the mRNA encoding amino acid residues #4 through #8 of H2A.Z and H2A.F. A 14-base long synthetic probe mixture with 32 different possible sequence combinations was designed and commercially synthesized. Both this probe and the previously utilized 14-mer probe mixture were used to make ^{32}P-labeled cDNA probes and each of these probes were used to probe duplicate nitrocellulose replicas of the human genomic-lambda library. The autoradiographs of the filters from each plate were aligned to see which plaques showed positive hybridization to both of these independent probes. Although many plaques were doubly-positive, a smaller fraction appeared to match quite well in signal intensity. The plaques corresponding to the best matches were isolated, the phage was grown, the DNA was isolated, digested with restriction enzymes, run on agarose gels, and southern-transfered onto nitrocellulose. Nitrocellulose filters made from transfer of DNA from duplicate gels were probed separately with the two ^{32}P-labeled cDNA's of forty lambda clones tested, five clones contained similar patterns of restriction fragments labeled by hybridization to each of the ^{32}P-cDNA's.

It therefore appears that human-lambda DNA clones have been found which hybridize selectively to two independent probes for the H2A.Z gene.

This project is now at the crucial stage of determining whether or not the DNA of the chosen human-lambda clones can be used successfully to hybrid select and in vitro translate the mRNA encoding H2.Z.

Significance:

Isolation of histone genes of the H2A family would permit studies into the molecular basis of the different regulation of histone variants relative to DNA replication.

Proposed Course:

1. To test the doubly positive clones with an H2A.F gene (probably related to H2A.Z) from chicken (donated by Prof. Julian Wells.)

2. To construct a synthetic probe using recently developed technology of inserting inosine into wobble positions. A longer probe could be constructed which could be used to search a genomic library directly.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06171-01 LMPH

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Chromatin Synthesis and the Control of Cell Proliferation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: William Bonner Head, Chromosome Structure and Function Section LMPH NCI

Others: Roy S. Wu Cancer Expert LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

Chromosome Structure and Function

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

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.)*

Our objective is to understand the relationship of various cell states and the transitions between them as reflected in the control of histone and chromatin biosynthesis. One phenomenon which we have studied is the differential sensitivity of the growth and chromosome cycles to the rate of protein synthesis. The chromosome cycle seems to be relatively invariant even though other results show that DNA synthesis is inhibited in parallel to the inhibition of protein synthesis. To resolve this apparent paradox, we studied histone and DNA synthesis under different conditions of protein synthesis and cell growth.

We have found and characterized a phenomenon which we have named chromosome cycle compensation. As protein synthesis is inhibited, the mRNA level of S-phase histones rises to compensate for the inefficiency of protein synthesis. There is evidence in the literature that normal and tumorigenic cells may differ significantly in their level of chromosome cycle compensation. Understanding this mechanism may lead to some insights into the growth characteristics of normal and tumorigenic cells.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Introduction:

The biochemical mechanisms which regulate cell growth remain largely unknown. For example it has been widely documented that as cell growth is slowed, the chromosome related activities and cell cycle phases are relatively unaffected. The biochemical basis for the phenomenon was unknown. Our studies this year have resulted in an explanation for this phenomenon in CHO cells.

Objective:

1) The development of a greater understanding of the relationship of the mechanisms regulating chromatin biosynthesis to the molecular mechanisms regulating cell proliferation.

2) A characterization of the responses of these regulatory mechanisms to the development of new regimens or compounds that might control inappropriate proliferation of transformed cells.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional eletrophoresis. (Methods developed in this laboratory).

(2) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(3) Maintenance of cells and nuclei in viable non dividing states using modified and defined media.

(4) Isolation and analysis of mRNA from different parts of the cell cycle or from quiescent cells.

(5) FACS analysis of cell cycle distributions including the BrdU antibody technique for measuring S phase cells.

Major Findings and Accomplishments:

Mechanism for the Relative Invariance of the Chromosome Cycle

The information reported in the literature concerning the regulatory mechanisms between DNA and protein synthesis has generally been obtained using high concentrations of inhibitors which slow either DNA or protein synthesis to 1-5% of their control level and which prevent cell growth.
We decided to investigate the reaction of cells to lower concentrations of inhibitors of DNA and protein synthesis, concentrations which still allowed cell growth. We have found that when cells are treated with concentrations of cycloheximide that inhibit protein synthesis by about 50%, the rates of DNA and histone synthesis are immediately inhibited by approximately 50%, but then recover over the next two hours to rates which are close to their control rates. The recovery is selective for S-phase histone synthesis compared to the

synthesis of nonhistone proteins and certain basal variants (i.e. H2A.X and .Z).

These findings suggest that proliferating CHO cells favor replication related synthesis when total protein synthesis is inhibited. We have found several observations in the literature which support our findings. It is known that cells grow exponentially in low concentrations of cycloheximide, but with increasing doubling times. For example in some cell types one can inhibit protein synthesis by almost 50% without lengthening S-phase. Our findings of a recovery of histone and DNA synthesis after inhibition of protein synthesis provides a biochemical basis for this observation.

This differential sensitivity of G1 and S-phase to the rate of protein synthesis has usually been interpreted in terms of a specific sensitivity of G1. Our results show that this explanation is not completely correct, and that explanation is that the synthesis of proteins related to the chromosome cycle is increased to counteract the overall inhibition of protein synthesis.

Cycloheximide slows the elongation step of translation and as such may not be a natural method of regulating protein synthesis. We are studying other more physiological methods of limiting protein synthesis. One method which we have preliminary data for is the isoleucine starvation of CHO cells. When put into isoleucine-lacking media, CHO cells finish S-phase and accumulate in a G0 state, according to their histone synthesis pattern. The histone mRNA level elevates soon after introduction of the CHO cells into the isoleucine-minus media, then decreases.

Significance:

There are suggestions in the literature that normal and tumorgenic cells respond very differently in terms of cell cycle distribution when their growth is slowed. Our findings provide a molecular basis for this effect and thus provides experimental framework for studying this phenomenon.

Proposed Course:

1. To characterize the effect of slow growth on the chromosome cycle in several cell lines, particularly matched pairs of normal and transformed lines, such as mouse 3T3 and BP-3T3 or human IMR-90 and VA-13.

2. To test whether chromosome cycle compensation exists in these cells or whether there are significant differences.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06172-01 LMPH

PERIOD COVERED

October 1, 1984 to September 30, 1985

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

Base Sequence Selective DNA Alkylation Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kurt W. Kohn	Lab. Chief	LMPH NCI
Others:	William B. Mattes	Staff Fellow	LMPH NCI
	John A. Hartley	Visiting Fellow	LMPH NCI
	Ronald Rahn	Guest Researcher	
	Judith Markovits	Guest Researcher	LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.2	3.0	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.)*

A new project was undertaken to investigate the DNA base-sequence selectivity of alkylation reactions. The main objective is to determine whether base-sequence selectivities contribute to the anti-tumor activities of alkylating agents and whether the effectiveness of these drugs could be enhanced by structural modifications that would optimize the selectivity for certain sequences. A second objective is to utilize alkylation reactions to probe DNA structure and conformation in solution, especially with reference to the major groove. A DNA fragment being utilized in these studies contains the 5'-flank of the human c-H-ras-1 oncogene which contains a very GC-rich region, a possible hot-spot for alkylation reactions. DNA sequencing methodology was used to localize alkylations at guanine-N7 positions. Marked differences in reaction intensities were observed at different guanines in a DNA sequence, as well as between different nitrogen mustards. Some major sequence-dependent patterns were observed and are being investigated quantitatively.

GPO 914-918

Project Description:

Objectives

1. Determine the base sequence selectivities for the reactions of alkylating agents with DNA.

2. Investigate the structural and conformational dependences of the selectivities.

3. Devise new alkylating agent structures with enhanced base sequence selectivities, especially those targeted for certain oncogenes.

Methods

1. High-resolution polyacrylamide gel electrophoresis of end-labeled DNA restriction fragments previously reacted with alkylating agents and chemically cleaved at sites of guanine-N7 alkylation.

2. Quantitative densitometry and computer analysis of electrophoretic gel autoradiograms.

3. Determination of interstrand crosslinking of restriction fragments by DNA denaturation and selective enzymatic degradation of single-strands.

4. Analysis of binding of [^{195m}Pt] cisplatin to DNA restriction fragments.

Major Findings:

Reaction of Nitrogen Mustards at Guanine-N7 Positions

We began with the hypothesis that, since alkylating agents react predominantly with guanine in DNA, a significant aspect of the biological action of the drugs could be due to selective reaction at GC-rich regions of the genome. In order to determine where highly GC-rich regions may occur in the human genome, a computer search of the DNA sequence data base at NIH was carried out. The search was for segments in which at least 17 of 20 bases were G or C. Among the genes retrieved, several oncogenes were represented, most notably H-ras which has an extraordinarily high GC region in its 5'-flank. We therefore are focusing our study on the plasmid pbc-N1 which is pBR 322 with a human c-H-ras-1 insert. We have prepared a restriction fragment of pbc-N1 containing pBR sequences and the adjacent very GC-rich (85% GC) 5'-flank of c-H-ras-1. The initial studies however were conducted using pBR 322 not containing the insert.

The major findings to date are the following: (1) there are marked differences in reactivity among guanines in a DNA sequence; (2) the differences are not markedly dependent on the solvent conditions of reactions; (3) tne guanine selectivity pattern is different for different nitrogen mustards; (4) the potential inter-strand crosslinking sites -- e.g. 5'-GC-3' -- show relatively low reactivity for HN2, melphalan and spirohydantoin mustard; (5) these sites however show a markedly enhanced reaction with uracil mustard; (6) runs of G's (potential sites of intra-strand crosslinking) exhibited disproportionately high

reactivities; (7) quinacrine mustard exhibited a uniquely different pattern of reactions.

Reaction of Chloroethylating Agents at Guanine-N7 Positions

Similar experiments were conducted using 2-chloroethyl(methylsulfonyl)methanesulfonate (ClEtSoSo), cis-2-OH-CCNU and mitozolomide. As described in another report, these compounds chloroethylate (and in some cases hydroxyethylate) DNA. We wanted to compare these compounds for selectivity for reaction. In these studies only reaction at guanine-N7 was observed, although it will be of interest to devise methods for observing reaction at guanine-O6.

The major findings were that cis-2-OH-CCNU exhibited marked differences in reaction intensities with different guanines. Especially striking were the disproportionately strong reactions at runs of 4 or more G's. ClEtSoSo differed from all other alkylating agents so far studied, in that the reaction intensities for all G's appeared to be the same.

In view of this uniform reactivity and also of the bulky structure of the molecule, ClEtSoSo may be useful as a foot-printing reagent, especially for the major groove.

The results indicate that ClEtSoSo differs significantly from the other 2 chloroethylating compounds in the base sequence-selectivity of its reaction with DNA.

Selective Binding of Cisplatin in DNA

The relative binding of [^{195m}Pt]-cisplatin to restriction fragments of pbc-N1 was studied. Marked differences were observed in binding to the different restriction fragments, even after normalization for guanine content. The very GC-rich regions of c-H-ras-1 exhibited especially intense binding of cisplatin. This again suggests that highly GC-rich regions may be significant targets for DNA-reactive anti-cancer drugs.

Significance:

1. These studies may lead to the development of new alkylating agents with high base-sequence selectivities, especially for certain oncogene sequences.

2. The studies may also yield new information about the structure and conformations of DNA in solution.

Proposed Course:

1. Carry out quantitative densitometry and computer analyses of the sequence-dependence of the reactions of a wide variety of nitrogen mustards.

2. Carry out molecular graphics analyses in order to obtain structural hypotheses to account for the observed selectivities. Test these hypotheses by synthesis of new nitrogen mustard structures that would be predicted to show strong selectivities.

3. Study congeners of ClEtSoSo currently being synthesized at Southern Research Institute in order to determine whether sequence selectivity can be restored to this class of compounds.

4. Devise a method to measure reactions at guanine-06 positons.

5. Extend these studies to DNA in chromatin and intact cells.

Publications:

None

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1984 - September 30, 1985

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.

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 Biometric Research Branch (BRB) provides statistical consultation to the other branches of CTEP, to the extramural and some intramural activities of other programs in DCT, and to the statistical centers of the clinical cooperative groups. It also carries on research in statistical methodology relating to cancer clinical trials.

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 and Information Office. The Protocol and Information 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. Edwin T. Jacobs retired from Government service; Drs. Silvia Marsoni, Mercedes Lassus, Elihu Estey, and Elizabeth Read left the program for positions elsewhere, to be replaced by Drs. Bruce Cheson, Hoo Chun, Gisele Sarosy, and F. Andrew Dorr. Drs. Michael Stewart and J. Michael Hamilton also joined the staff of CIB. Dr. Michael Hawkins was recruited from the University of Wisconsin as Head, Biologics Section in IDB. Dr. Maryann Roper also joined the staff of the Biologics Section. Dr. Lawrence V. Rubinstein joined the staff of the BRB, where Dr. Frances J. Mather of Tulane University spent a sabbatical year and Dr. Sate Songorabadi served as a Visiting Fellow. Dr. Brigid Leventhal, on sabbatical from the Johns Hopkins University, served as Special Assistant to the Associate Director.

III. Highlights in Program Development

A) Immunodeficiency Syndrome (AIDS)

Over the past year, staff members of the CTEP have been heavily involved in activities related to AIDS. Dr. Killen has chaired the NCI committee coordinating extramural support for AIDS research. This group works closely with other components of the NIH actively involved in supporting AIDS research. 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. 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 characterize further the nature of the viral infection with the HTLV-III family. Efforts along these lines have been aided immeasurably by the extensive network of collaboration set up between extramural investigators and the Laboratory of Tumor Cell Biology, DTP, DCT, NCI (see the Report of the Associate Director for the Developmental Therapeutics Program).

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 one 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 investigations of health care workers, prisoners, etc.

Therapy - During the past year the leading hypothesis in therapy has been generated by Broder's observation that Suramin, an inhibitor of retroviral reverse transcriptase, also inhibits HTLV-III replication in vitro. Broder has also shown that Suramin treatment of AIDS patients results in a fall of lymphocyte-associated reverse transcriptase levels. In response to this exciting development, CTEP has coordinated a multicenter working group for the further testing of Suramin in patients with AIDS and ARC. Participating investigators are affiliated with most of the premier institutions involved in the AIDS epidemic. The clinical trials will be accompanied by clinical pharmacologic studies of Suramin and correlative viral studies.

B) Development of New Agents

Highlights of the year include:

1. Introduction of 15 New Agents into Phase I Trial: Of particular interest will be results with flavone acetic acid, an agent with broad solid tumor activity in preclinical testing, and Suramin, the first agent introduced into clinical trial as an antiviral agent for the treatment of AIDS. Among the biologics three monoclonal antibodies have begun clinical testing.

2. Drugs Entering Phase II: The following drugs entered Phase II trials during the year: pibenzimol, trimetrexate, taxol, caracemide, acodazole, and the radiosensitizer SR-2508.

3. Creation of a Biologics Evaluation Section: During this past year IDB began a systematic approach to the development of a Phase II and III program with biologic agents. The BES undertook a comprehensive review of the current status of the interferons, and as a result has targeted chronic myelogenous leukemia, hairy cell leukemia, and non-Hodgkin's lymphoma as specific areas for large-scale comparative trials. In addition the staff is organizing a definitive trial of the role of BCG in the treatment of superficial bladder cancer.

4. Important Clinical Trials Initiated: During the past year several clinical trials are worthy of special note. The Phase I program with Suramin is the first full-scale test of an agent specifically directed against the HTLV-III virus.

   HMBA is a polar planar compound that selectively differentiates Friend erythroleukemia and HL60 cells in vitro at non-cytotoxic doses. This drug is now well into Phase I trials; doses of 19.2 g/m^2 given as a continuous infusion over 5 days yield plasma levels over 1 mmolar, the concentration necessary to induce differentiation in the in vitro models. Since this effect is dose-dependent in vitro, Phase I trials are continuing in order to escalate the dose further.

   Extensive plans have been drawn up for two active platinum analogs, CBDCA and CHIP in ovary cancer, small cell carcinoma of the lung, and head and neck

cancer, in order to define the roles of these drugs in initial therapy. Randomized scheduling trials with trimetrexate in head and neck cancer and possibly breast cancer are being arranged at the present time. Ifosphamide has been shown to have significant activity in adult soft tissue sarcoma, and three randomized Phase III trials (intergroup sarcoma, NCI-C, and ECOG) are currently being developed to define its role in combination with doxorubicin with or without DTIC in initial therapy. The role of homoharringtonine in combination therapy (especially with VP-16) is still be determined in acute myelocytic leukemia. Similarly the role of deoxycoformycin ± interferon is being determined in hairy cell leukemia. A randomized Phase III trial of non-cross resistant regimens including the M.D. Anderson MIME regimen is now being negotiated with M.D. Anderson and Memorial Hospitals.

Phase II clinical trials are continuing on 35 cytotoxic drugs.

5. Identification of drug activity: Plans for the platinum analogs CBDCA and CHIP have been noted above. Homoharringtonine is active in acute leukemia; hypotension, previously the dose-limiting toxicity, is manageable if the drug is given as as a slow infusion. The activities of AZQ in brain tumors, AMSA in acute leukemia, and mitoxantrone in breast cancer are being further defined in randomized controlled trials against BCNU as a single agent, daunorubicin in combination, and doxorubicin, respectively. Phase II data have suggested that PCNU may be superior to BCNU in the treatment of brain tumors; Phase III comparative trials are just beginning.

6. Closing of IND's: IND's for maytansine and trifluorothymidine were closed because of a lack of clinical activity.

7. Areas of Special Emphasis: A Request for Application (RFA) to study in depth certain areas in biochemical modulation will shortly be issued. An RFA for the study of differentiation agents is in preparation.

8. Liaison with European Clinical Drug Development: During the past year major efforts were continued to develop collaborative liaisons with the components of the EORTC and the Cancer Research Campaign in Great Britain. The goal is to create a two-way flow of drugs across the Atlantic with minimum time delay. This effort also seeks to reduce unnecessary duplication of trials which had occurred in the past because investigators often wished to (or needed to) reproduce the results of a reported study in their own country.

   This renewed effort is a response to the increased efforts devoted to cancer drug development in Europe. NCI is interested in the possibility of bringing promising drugs into the U.S. after early clinical trials in Europe. In addition to promoting increasing attendance of American and European investigators at meetings of mutual interest, the DCT is supporting an increased level of monitoring for certain high priority Phase I trials in Europe.

9. Reduction of drug cost: Emphasis on improved management practices and tighter protocol review during the past year continue to yield savings in the cost of clinical drugs. We estimate a decrease of about $1.4 M in FY '84 compared with FY '83.

10. Development of Case Report Forms and Study Summaries: To enhance the value of CTEP's data base on the clinical activity of experimental agents, standardized case report forms have been developed for use by the drug development contractors. In addition, standardized study summaries will be required for all trials using experimental drugs performed in cancer centers. These summaries will greatly increase the reliability and completeness with which trials results are reported to CTEP and will improve the management of the clinical drug development program.

11. Site Visit Monitoring: All 19 cooperative groups supported by the Cancer Therapy Evaluation Program now have active monitoring programs administered by the groups themselves under CTEP oversight. Since 1982 a total of 475 member institutions and 465 affiliate institutions have been audited; during the current year 114 members, 66 affiliates, and 45 CCOPs were site-visited.

    The Quality Assurance and Compliance Section directly oversees the monitoring of Phase I and cancer centers studies. Phase I studies are monitored three times per year. Since 1982, 43 cancer centers site visits (involving 33 institutions) have been accomplished. Sixteen were carried out in the current year.

C) Group Studies

Noteworthy contributions are being made by the clinical cooperative groups in several areas, and interesting new initiatives by the groups are in progress. Early results from the NSABP's clinical trial comparing modified radical mastectomy to breast-preserving surgery demonstrates that the lesser surgery plus radiotherapy is as effective as conventional primary treatment in preventing recurrence. Additional followup is necessary. The GITSG has completed a controlled trial indicating benefit for the use of adjuvant radiotherapy plus chemotherapy compared to surgery alone for patients with resectable colorectal cancer. POG has recently conducted a randomized trial in osteosarcoma which conclusively demonstrated the benefit of postoperative adjuvant chemotherapy over a non-treatment control. This trial addressed a controversial issue whose clarification was essential for optimal patient care, as well as for future studies in osteosarcoma. CALGB has active, group-wide studies of the lymphoproliferative disorders including sophisticated immunological and cytogenetic evaluations and therapy including promising new agents such as interferon and deoxycoformycin. SWOG is planning a large Phase III comparison of two of the newer, more aggressive chemotherapy regimens for non-Hodgkin's Lymphoma with CHOP, long considered "standard" therapy by many practicing oncologists. In a study of women with advanced breast cancer, MAOP has initiated the first groupwide trial involving response-surface methodology; successful use of these statistical and mathematical techniques may permit the development of a much more rational approach to the construction of optimal drug combinations. CCSG has demonstrated that routine histopathologic classification of non-Hodgkin's lymphoma is the best criterion for choice of therapy for patients with generalized involvement. The National Wilms' Study #3 suggests that tumors designated "favorable histology" can be successfully treated with less intensive regimens. Long-term survival rates of 90% have been demonstrated for patients with non-metastatic favorable histology Wilms' Tumor. CCSG has demonstrated that elevation of serum ferritin levels in neuroblastoma is associated with a significantly poorer prognosis than was normal ferritin and that this correlation is independent of stage and age

at diagnosis. Breast adjuvant chemotherapy studies ongoing in three cooperative groups (NSABP, SEG, CALGB) are exploring regimens that are substantially more aggressive in dose intensity than therapies tried over the past decade.

The increasing participation of two or more cooperative groups in a single trial is a laudable trend that we have been actively promoting. Examples of such intergroup studies organized during the past year include:

1. Intergroup Prostate Study: This trial will attempt to confirm impressive preliminary results suggesting that the combination of an LHRH agonist (leuprolide) with an antiandrogen (flutamide) yields much better results than the LHRH agonist alone.

2. Intergroup Colon Adjuvant Trial: This study is designed to replicate an earlier, smaller trial conducted by the NCCTG. Preliminary analysis suggests that patients receiving 5FU plus levamisole following curative surgery for colon cancer may have fewer recurrences than those treated with surgery alone.

3. Head and Neck Intergroup Study: Patients with localized, advanced disease will be treated in a multimodality study, testing the hypothesis that three cycles of cisplatin plus 5FU adds significantly to the efficacy of standard local therapy.

Several ongoing intergroup efforts continue, including:

1. The Intergroup Rhabdomyosarcoma Study Group has developed a prognostic classification system based on cytohistologic subtypes that identifies those patients who require more intensive therapy.

2. Intergroup Soft Tissue Sarcoma Study: Patients with primary soft tissue sarcoma (STS) after definitive surgery and/or radiation therapy are randomized to either high-dose intermittent doxorubicin adjuvant therapy or a control arm of no further therapy. Most major national and regional groups participate.

3. Intergroup Mesothelioma Study: Patients with limited unilateral thoracic mesothelioma receive combined surgery and radiation therapy and are randomized to receive adjuvant doxorubicin or a control arm. Several major national groups participate.

4. Intergroup Melanoma Study: Patients with intermediate thickness primary melanoma are randomized to have an excision with a 2 cm versus 4 cm margin and to have elective lymph node dissection or not. All cooperative groups participate as well as the NCI of Canada and the Melanoma Clinic, Australia.

E) Cancer and Nutrition Program

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 examining 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. Ota et al are studying the effect of intravenous nutritional support on the cellular kinetics of rectal carcinoma. Patients are being randomized either to receive immediate surgery or to receive one week of preoperative hyperalimentation. Biopsy specimens collected prior to and during surgery will be analyzed for cellular growth parameters. This study will help to define the role of nutritional support in the cancer patient.

F) NCI-Pan American Health Organization: Collaborative Cancer Treatment Research Program (PAHO:CCTRP)

The collaboration between U.S. and Latin American investigators in the development of clinical studies continues to be the major goal of this program. An extensive re-evaluation of goals has resulted 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 cancers; 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 establishment of a scientific advisory committee to coordinate scientific directions, to review critically protocols and publications, and to evaluate membership status; 4) assess feasibility and scientific merit of proposed Phase III studies prior to submission to NCI. These will preferably be performed by more than one center to ensure adequate accrual. More effort 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 group to make unique contributions.

G) Clinical Trials Review

CTEP has embarked upon an ambitious attempt to review the effectiveness of the clinical trials mechanism sponsored by the NCI. Initially the review will focus upon the performance of the cooperative groups during the time interval 1979-1984. The reviews will be disease-oriented and will be conducted by small committees of experts selected by CTEP. For each disease category the reviews will attempt to answer the following questions: 1) What were the most pressing clinical research issues in cancer treatment during the specified time period? 2) Were these issues dealt with adequately by the cooperative groups? 3) Was there an undesirable degree of duplication of activity in the cooperative group system? 4) What was the quality of the clinical trials methodology employed by the groups, with specific reference to the design, execution, analysis, and reporting of trials over the five-year period of interest?

Thus far the committees for testis cancer and non-small lung cancer have met, concluded deliberations, and are in the process of preparing reports. The review will continue with many other disease sites and with certain areas of categorical

concern, such as the drug development program. We anticipate that the results of the review will be extremely important in modifying our approach to the organization and coordination of clinical trials in the coming years.

H) Organization and Coordination of Clinical Trials

With increasing opportunities for clinical implementation of exciting new developments in the laboratory, the cooperative groups face a future full of bright possibilities. Since the likelihood of a relatively flat budget is fairly strong for the near future, the size of the clinical trials mechanism cannot be expected to increase significantly. For these reasons selection and prioritization of questions suitable for clinical trials are perhaps more important than ever before. In addition, experience over the past few years strongly suggests that the size of certain kinds of trials, particularly comparative trials where survival is a major endpoint, should probably be much larger than has been customary in the past. All these observations point to the need for an increasing level of coordination among clinical trials groups, probably at the national level.

CTEP has begun discussion with the cooperative group chairmen about ways to achieve these ends. During the next few months we plan to develop a system for identification, prioritization, and implementation of key questions in cancer therapy at the national level. Doing so on a broad scale may involve a substantial alteration in the way many cooperative groups do their business. The goal of achieving a maximally effective clinical trials program clearly justifies explorations along these lines.

I. Modification of the Guidelines for Peer Review of the Clinical Trials Groups

In connection with our anticipated increased emphasis on large trials involving the cooperation of several groups, it will be necessary to define carefully the terms according to which the cooperative groups are to be reviewed. Traditionally the peer review process rewards accrual and the generation of studies by a group. If the number of trials is to decrease (with the advent of larger trials), groups will have to be evaluated, at least in part, on the effectiveness of their participation in high-priority studies, whether or not the study was generated within the group. We also wish to replace the overemphasis on raw accrual figures with a more balanced assessment that will include strong attention to the quality of the ideas submitted to clinical trial by a group. Accordingly we have begun to rewrite the guidelines for cooperative group review with these and the other similar issues in mind.

J. Cost of Clinical Trials

As part of our attempts to rationalize the process of budgeting for clinical trials, we have determined that a systematic examination at the resource needs of the various types of clinical trials is necessary. A working group within CTEP, chaired by Ms. Kim Horgan (Program Analyst, OAD), is attempting to develop methodology to define these resource requirements. We expect that methodology will be set by the end of FY '85. The project will then enter a phase of data gathering. Having a more precise knowledge of the costs of clinical trials will make budgeting for them more rational and therefore more consistent across the clinical trials groups.

K. Representation in International Activities

CTEP is responsible for providing clinical input into various international agreements adminstered by DCT. The PAHO Treatment Research Programs have already been mentioned, as have the increasingly close links with the European Organization for Research on the Treatment of Cancer (EORTC) which has had for several years a strong link with NCI. A member of the EORTC attends the Phase I Working Group meetings in Bethesda; CTEP staff is represented on the Protocol Review Committee of the EORTC. CTEP staff also provides clinical input into the bilateral agreements between the U.S. and France, Italy, Germany, and Japan. Major emphasis in these agreements thus far has been on new drug testing.

L. Analyses in Progress

In its role as coordinator and sponsor of most NCI-supported clinical trials of cancer therapies, CTEP has the important responsibility of assessing the suitability of current approaches and methodologies. Among the many questions currently under investigation by CTEP staff are:

1. How accurately do the findings in preclinical toxicology predict (a) a safe starting dose in Phase I trials, and (b) the pattern of organ toxicities seen in the clinic?

2. To what extent can therapeutic responses seen in Phase I trials predict subsequent activity in Phase II?

3. How accurately has the NCI's preclinical tumor panel predicted activity of new agents in human cancer?

4. Have clinical adjuvant chemotherapy trials performed over the past ten years provided adequate tests of the hypotheses generated in laboratory models? To what extent can deviations from the laboratory models explain the borderline or negative results of many clinical trials?

5. What are maximally efficient study designs for assuring that promising new therapies are not overlooked, and therapies which do not offer significant advantages over standard treatment are discarded after entry of the minimum number of patients?

6. How may the concepts of biochemical modulation be most effectively applied and tested clinically?

7. How may the concepts and techniques of statistical inference be extended or adapted to deal with such problems as the early termination of clinical trials, crossover interactions between treatment efforts and patient characteristics, and estimating the proportion of total variability in outcome explained by prognostic factors with censored survival data?

M. Sponsorship of Meetings and Workshops

In addition to the usual meetings of the new drug development contractors and the Biochemical Modulation Advisory Group and the numerous working sessions with extramural investigators necessary to carry out the programs mentioned above, several other meetings were of particular interest:

1. National Symposium on Methodology and Quality Assurance in Cancer Clinical Trials

   This major national two-day symposium was organized and sponsored by the Biometric Research Branch to stimulate improvement in the methodology of cancer clinical trials. The symposium was attended by almost 300 investigators from cancer centers, cooperative groups and the pharmaceutical industry. The full proceedings will be published in Cancer Treatment Reports. Planning for a 1986 symposium on the same topic is underway.

2. In connection with CTEP's effort to promote clinical research activity in surgical oncology, a workshop on adjuvant therapy after resection of isolated hepatic metastasis from colorectal cancer has been held and a protocol is being developed. A second workshop dealing with surgical quality control in the cooperative groups is to be held in July 1985 and two other workshops looking at adjuvant therapy to surgery in the treatment of hepatomas and rectal cancers are in the planning stage.

3. As part of IDB's efforts to develop uniform toxicity criteria, cooperative group representatives met to discuss a draft set of criteria. We plan to push hard to establish common criteria, which would do much to enhance the interpretability and comparability of trials conducted by different groups.

IV. Publications

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5. Magee, M.J., Howard, J., Bosl, G.J., and Wittes, R.E.: Phase II trial of 4'-epidoxorubicin in advanced carcinoma of head and neck origin. Cancer Treatment Reports 69:125-126, 1985.

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1. Statistical Planning and Scientific Monitoring of NCI Sponsored Extramural Clinical Trials

A statistical review is performed of all NCI sponsored extramural clinical trials. The BRB attempts to ensure that such trials are planned, conducted and reported in a sound statistical manner. The BRB is particularly active in the planning and monitoring of inter-group trials. Trials which have received particular attention during the past year include: National Prostatic Cancer Project trials for the adjuvant treatment of prostatic cancer patients; planning of national prospective trial for limb perfusion of stage I-II melanoma patients; planning of multi-institution retrospective analysis of limb perfusion for stage III melanoma patients; planning of national prospective trial for chemotherapy of patients who have had resection of isolated hepatic metastases; Pediatric Oncology Group trial of adjuvant chemotherapy for patients with osteosarcoma; Intergroup trial of adjuvant 5-FU plus levamisole for colorectal cancer; Jamaica studies of the chemotherapy of HTLV associated T-cell lymphomas; Intergroup study of pediatric Hodgkin's disease; Intergroup studies of the treatment and histologic classification of rhabdomyosarcoma; Cancer and Leukemia Group B studies of relapse treatment for adult leukemia; M.D. Anderson studies of first line treatment for adult leukemia; Piedmont Oncology Association trial of tamoxifen versus megace for the treatment of metastatic breast cancer; planning multi-center trials of high dose chemotherapy with autologous bone marrow rescue in a variety of solid tumors.

The BRB serves as liaison to extramural statistical centers and makes recommendations concerning the funding of statistical activities. The branch conducted a programmatic site visit of the Southwest Oncology Group Statistical Center in Seattle in order to provide a basis for a critical funding decision. The BRB participates in the organization and funding of meetings and workshops of statisticians involved in major extramural cancer clinical trials in order to improve statistical and data management procedures for the conduct of these studies. A meeting of cooperative group statisticians was held in New Orleans in May 1985 and a meeting of statisticians, data managers and executive officers is currently being planned for late 1985 to develop revised guidelines for the conduct of Intergroup studies.

2. Collaborative Clinical Research

The Biometric Research Branch staff provide statistical collaboration on the following clinical studies. Principal clinical collaborators are listed in parentheses.

a. National clinical trials of the staging and treatment of early ovarian cancer (Dr. Robert Young).
b. Multi-institution randomized clinical trials of nutritional support in the treatment of patients with solid tumors (Dr. Daniel Nixon).
c. Multi-institution clinical trials of chemotherapy in advanced unresectable head and neck cancers (Dr. Mario Eisenberger).
d. Multi-institution clinical trials of surgery and adjuvant chemotherapy in resectable lung cancer (Dr. Carmack Holmes).
e. Multi-institution clinical trials of magnetic resonance imaging (Dr. Ruzicka).

f. Intramural clinical trials of the Biological Response Modifier Program.
g. Intramural antiemetic clinical trials of the Surgery Branch.
h. National Registry of colorectal cancer patients who have had resection of hepatic metastases. This national registry currently contains about 700 cases and is used to distinguish the kinds of patients who do well or poorly after hepatic resections (Dr. Sugarbaker).
i. Review of results in the chemotherapy of advanced gastric cancer (Dr. Killen).
j. Review of survival results in the chemotherapy of advanced prostatic cancer.
k. Review of results with hexamethylmelamine in advanced ovarian cancer (Dr. Foster).
l. Comparative analysis of functional outcomes in women undergoing mastectomy or excision plus radiotherapy for primary breast cancer (Dr. Gerber).
m. Analysis of prospectively collected psychosocial data from a randomized trial of mastectomy versus excision plus radiotherapy for primary breast cancer (Dr. Schain).
n. Planning the comparison of histologic classification systems for rhabdomyosarcoma (Dr. Ungerleider).
o. Evaluation of results in the treatment of 1900 patients in phase I trials to determine the extent to which phase II drug activity can be predicted (Dr. Marsoni).
p. Comparison of children and adults with regard to toxicities and maximum tolerated doses of chemotherapeutic agents (Dr. Marsoni).
q. Evaluation of prognostic significance of pathologic and clinical heterogeneity of lymphocyte depleted Hodgkin's disease (Dr. Elaine Jaffe).
r. Analysis of sperm counts in men taking 13-cisretinoic acid for skin disorders (Dr. DiGiovanna).
s. Analysis of prognostic factors for patients with colorectal cancer. The St. Mary's Hospital (England) data base of over 20,000 cases is being used for these analyses (Dr. Fielding).

3. Preclinical Drug Discovery

a. The BRB is actively collaborating with the Developmental Therapeutics Program in the statistical design of screening procedures using a non-clonogenic assay with human tumor cell lines. We are also developing methods for the analysis of histologic specificity of results from the colony forming assay or the new assay. Computer programs have been written by the BRB that implement two methods for assessing histologic specificity and research on a third improved method is underway. These methods have been used to evaluate histologic specificity for over 60 compounds that have undergone stage 2 testing in the colony forming assay against fresh human tumors. Results were reported at AACR. Methods under development for the design and analysis of the new non-clonogenic assay emphasize estimation of log-cell-kill and determination of activity and dose-response using procedures with sound statistical bases.
b. Evaluation of the 1975 "tumor panel experiment" in collaboration with Drs. Sylvia Marsoni and Daniel Hoth and investigators of the Developmental Therapeutics Program.
c. Development of methodology for analysis of tumor growth curves for use in analysis of biological response modifier pre-clinical screens (Dr. Talmadge).

d. Development of improved statistical designs for screening compounds using the P388 in-vivo system. This research was conducted in collaboration with Drs. Paull and Hodes.

4. National Symposium on Methodology and Quality Assurance in Cancer Clinical Trials

This major national two day symposium was organized and sponsored by the Biometric Research Branch. It was very successful in stimulating improvement in the methodology of cancer clinical trials. The topics were very broad ranging and the symposium was attended by almost 300 investigators from cancer centers, cooperative groups and the pharmaceutical industry. The full proceedings are being edited by the Biometric Research Branch and will be published in Cancer Treatment Reports. Planning for a 1986 symposium on the same topic is underway.

5. Development of Methodological Guidelines for the Publication of Reports of Cancer Clinical Trials

These guidelines were developed in collaboration with Dr. Robert Wittes and adopted by the editorial board of Cancer Treatment Reports. They constitute nine specific recommendations that appear in every issue of CTR which can substantially improve the quality of clinical trial reports. It is hoped that other journals will adopt these guidelines.

6. Evaluation of Cooperative Group Cancer Clinical Trials

This important project utilizes small teams of extramural experts on a disease oriented basis to evaluate the past 5 years of cooperative group cancer clinical trials. The teams address whether the most important questions are being asked and whether trials are being planned and conducted in a manner that yields reliable answers. The BRB is involved in the planning and conduct of this project which is well underway.

7. Investigator's Handbook

The BRB participated in writing parts of this important document dealing with the statistical design, monitoring and analysis of clinical trials.

8. CTEP - Information System

The BRB provided extensive consultation on the hardware and software alternatives appropriate for the effective development and use of this system. The BRB secured an outside consultant to participate in this process of developing an RFP for the procurement of appropriate hardware and software.

9. Development of methodology to evaluate the cost of various kinds of clinical trials and to evaluate the impact of funding patterns. This project is led by Kim Horgan.

10. Comparative Studies to Evaluate Magnetic Resonance Imaging

These multi-institution studies are being sponsored by the Radiation Research Program. BRB staff participated extensively in the design of these studies

in developing an estimate of requirements for coordinating center funding, and in providing statistical collaboration during their conduct.

11. Computer Software Development

a. Development of a microcomputer data base for the National Hepatic Metastases Registry. DBASE 3 on a Compaq Plus computer was used.
b. Development of an interactive computer program for the generation of survival curves on a graphics terminal. This is a very powerful improvement of a previous program. It is written in the SAS language and has been requested by several major statistical centers. A description of this program will be published.
c. Initiation of projects for the Small Business Innovative Research Program that would result in the development of computer systems for interactive analysis of data that would bring state-of-the-art methodology and interactive graphics to the disposal of statisticians, clinical investigators and bench scientists.
d. Development of interactive computer programs for use by BRB and Developmental Therapeutics personnel for assessing histologic specificity of new compounds in in-vitro assay against human tumors and cell lines.
e. Development of an interactive computer program to calculate exact confidence limits for response rates and response rate differences for use by CTEP staff.

12. New Methodology for Evaluating Chronic Toxicity as a Function of Cumulative Dose

Assessment of the relationship of acute toxicity to dose of an administered drug is usually straightforward. This is not the case for chronic toxicity such as irreversible cardiac or lung damage. Because some patients receive low doses due to early death and because extended survival may be necessary to observe clinical evidence of toxicity, the usual analyses are biased. We have developed new statistical methodology for such problems.

We are using these new methods to re-analyze the large data base of the Southwest Oncology Group concerning the relation between cardiotoxicity and cumulative dose of mitoxantrone. Unfortunately, the original data base used by Von Hoff for analysis of adriamycin cardiotoxicity is no longer available. The new methodology also has broad applicability to studies of second malignancies and to epidemiologic investigations of occupational health.

13. Testing for Qualitative Treatment by Subset Interactions

A qualitative interaction is said to exist involving two treatments (A and B) when treatment A is best for one subset of patients but treatment B is best for another subset. Many major clinical trials in cancer, cardiovascular diseases and other areas conclude with a report of qualitative interactions. Such claims are very important because of their implications on treatment selection for individual patients and because of their biological implications. We have, during the past year, developed the first valid statistical significan test of qualitative treatment by subset interactions. This test was used to re-analyze the National Surgical Adjuvant Breast Project's Protocol for the evaluation of tamoxifen.

These results will be published in the June 1985 issue of Biometrics. These results and other results concerning new methodology for subset analysis have been presented in invited talks before the Washington Statistical Society, Society for Clinical Trials and the National Heart, Lung, and Blood Institute's September 1984 Workshop on Subset Analysis.

We have recently generalized this method to test the hypothesis that some subsets of patients benefit from one treatment whereas for others the treatments are equivalent. This generalization was recently presented in conjunction with other results in an invited presentation to the Society of Clinical Trials on the topic of combining evidence across clinical trials. The new methodology is applicable to determining whether results from different institutions and different studies are "poolable."

This research was conducted jointly with Dr. Mitchell Gail. The research is continuing on other new methodology for subset analyses.

14. Modeling the Covariate Dependence of Binary Sequences

There is an increasing interest in studying quality of life of cancer patients on clinical studies. Psychological factors may be studied by treating them as dichotomous variables which vary over time between the states of "good" and "poor." We have explored modelling these transitions by means of a Markov chain, incorporating covariate dependence by means of a logistic model for the transition probabilities. We have applied these methods to a large study of the psychological effects of mastectomy. This research was conducted with Dr. Larry Muenz and has been published in Biometrics.

15. The Size of Cancer Clinical Trials

Many phase III trials are too small to identify therapeutic effects of the magnitude that can be realistically expected. The implications of having numerous phase III trials of inadequate size is also not generally appreciated. These results were presented at the November 1984 Symposium on Methodology and Quality Assurance in Cancer Clinical Trials and will be published in Cancer Treatment Reports.

An evaluation of the appropriate size of phase II trials is also being conducted. Preliminary results were given in invited presentations before the Washington Statistical Society, NYU Medical Center and at the Memorial Sloan Kettering Cancer Center.

16. Randomized Phase II Clinical Trials

An evaluation of several kinds of randomized designs for phase II clinical trials has been conducted. Although some proposed randomized designs seem of little value for phase II trials, the concept of randomizing among new agents for cooperative group trials in common diseases can be useful. The objectives of randomized phase II trials have been carefully assessed and distinguished from the objectives of phase III trials, and the use of ranking and selection theory for planning the size of such trials is developed. These results have been accepted for publication in Cancer Treatment Reports.

17. Methodology For Antiemetic Studies

A critical review of methodology used for the design, conduct and analysis of antiemetic studies in cancer patients was made in collaboration with Drs. Joseph Aisner and Ian Oliver. This manuscript has been submitted for publication.

18. Designs For The Early Termination Of Clinical Trials

We have continued research on methodology for early termination of randomized clinical trials when interim results for the experimental treatment are not promising. An easily utilized and effective two-stage design has been developed and presented at the International meeting of the Biometric Society and the November 1984 Symposium on Methodology and Quality Assurance in Cancer Clinical Trials. A manuscript dealing with the use of this design as an alternative to nonrandomized pilot studies in the treatment of head and neck cancers is in preparation.

19. Development of Methods For Treatment Planning Utilizing Mathematical Models

Clinical trials of the past 5 years were evaluated relative to the predictions of the "Norton-Simon model." These results provide support for the concept of intensification of active therapy. This review will appear in a special issue of Cancer Treatment Reports dealing with the interface between the laboratory and clinic.

20. Confidence Intervals For Differences In Response Rates

Research is being conducted to develop improved confidence intervals for the difference in proportions that are easy to calculate and have appropriate coverage probabilities. The increased use of confidence intervals in published reports could substantially reduce misinterpretation of results by both authors and readers. Two manuscripts on the new methods we have developed are in preparation.

21. Planning of Clinical Trial Strategies For Developing Effective Combinations

We have embarked upon a multi-faceted research project dealing with the development of new designs for sequences of clinical trials to identify effective combination regimens. We are pursuing the first portion of this exciting new area and the results are very encouraging. These initial results were described in invited presentations to the Cancer and Leukemia Group B, April 1985 meeting and the Southeastern Oncology Group's June 1985 meeting. The results are being prepared for publication.

22. Randomized Consent Designs

An evaluation of the randomized consent (pre-randomization) design has been completed. These designs were being used with increasing frequency though their properties are not well understood. The results of this analysis were

published in the N Engl J Med and presented to the Oncologic Drug Advisory Committee of the FDA as well as at plenary sessions of the Society of Clinical Trials and the Tucson Adjuvant Clinical Trials meetings. It appears that this study has had important impact on limiting the use of this problematic design.

23. Cost-benefit analysis of CEA monitoring in surgically "cured" colorectal cancer patients.

24. Evaluation of methodology for determining the required sample size for a clinical trial when time-to-event is the primary endpoint.

Publications:

1. Eisenberger, M.A., Simon, R., O'Dwyer, P.J., Wittes, R.E. and Friedman, M.A. A re-evaluation of nonhormonal cytotoxic chemotherapy in the treatment of prostatic carcinoma. Journal of Clinical Oncology (in press).

2. Ellenberg, S.S., Eisenberger, M.A.: An efficient design for phase III studies of combination chemotherapies. Cancer Treatment Reports (in press).

3. Ellenberg, S.S.: Randomization designs in comparative clinical trials. New England Journal of Medicine: 310: 1404-1408, 1984.

4. Feld, R., Rubinstein, L.V. and Weisenberger, T.H.: Sites of recurrence in resected stage I non-small cell lung cancer (NSCL): A guide for future studies. Journal of Clinical Oncology 2: 1352-1358, 1984.

5. Gail, M. and Simon, R. Testing for qualitative interactions between treatment effects and patient subsets. Biometrics (in press).

6. Gastrointestinal Tumor Study Group, The: Prolongation of D=disease free interval in surgically cured rectal carcinoma. New England Journal of Medicine: 312: 1465-1472, 1985.

7. Gerber, L.H., Helfgott, R.K., Gross, E.G., Hicks, J.E., Ellenberg, S.S., Peck, G.L.: Vertebral abnormalities associated with synthetic retinoid use. Journal of American Academy of Dermatology: 10: 817-823, 1984.

8. Lessner, H.E., Mayer, R.J., Ellenberg, S.S., Stablein, D.M.: Adjuvant therapy for colon cancer (Letter to the Editor). New England Journal of Medicine 311: 410, 1984.

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15. Shoemaker, R.H., Wolpert-DeFilippes, M.K., Kern, D.H., Lieber, M.M., Kamuch, R.W., Melnick, N.R., Miller, W.T., Salmon, S.E., Simon, R.M., Venditti, J.M. and Von Hoff, J.D.: Application of a human tumor colony forming assay to new drug screening. Cancer Research 45: 2145-2153, 1985.

16. Simon, R. The size of phase III cancer clinical trials. Cancer Treatment Reports (in press).

17. Simon, R., Wittes, R.E. and Ellenberg, S.S. Randomized phase II clinical trials. Cancer Treatment Reports (in press).

18. Simon, R. and Wittes, R.E.: Editorial: Methodologic Guidelines for Clinical Trial Reports. Cancer Treatment Reports 69: 1-3, 1985.

19. Smith, F.P., Ellenberg, S.S., Mayer, R.J., Lessner, H.E., Horton, J.B.: Phase II study of MOF-Streptozotocin (methyl-CCNU, vincristine, 5-fluorouracil and streptozotocin) in advanced colorectal cancer: a GITSG study. Journal of Clinical Oncology: 23: 770-773, 1984.

20. Sugarbaker, P.H., Vermess, M., Doppman, J.L. and Simon, R. Improved detection of focal lesions with computerized tomographic examination of the liver using ethiodized oil emulsion (EOE-13) liver contrast. Cancer 54: 1489-1495, 1984.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06308-14 BRB

PERIOD COVERED

October 1, 1984 through September 30, 1985

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

Biometric Research Branch

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard M. Simon, Chief, Biometric Research Branch, CTEP, DCT, NCI
Others:
Susan S. Ellenberg, Statistician, BRB, CTEP, DCT, NCI
Lawrence V. Rubinstein, Statistician, BRB, CTEP, DCT, NCI
Frances J. Mather, IPA, BRB, CTEP, DCT, NCI
Sate Songhorabadi, Visiting Fellow, BRB, CTEP, DCT, NCI

COOPERATING UNITS *(if any)*

Developmental Therapeutics Program, DCT, NCI; Radiation Research Program, DCT, NCI; Biological Response Modifiers Program, DCT, NCI; Clinical Oncology Program, DCT, NCI; Environmental Epidemiology Branch, DCE, NCI.

LAB/BRANCH

Biometric Research Branch

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	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 spece provided.)*

The Biometric Research Branch (BRB) is the statistical component for scientific planning and monitoring of the national and international research program of the Division of Cancer Treatment. The branch provides statistical leadership for all extramural activities of the division. The branch is also responsible for statistical consultation and collaboration with the intramural activities of the Biological Response Modifier Program, Developmental Therapeutics Program, and Radiation Research Program and performs collaborative research with components of the Clinical Oncology Program.

The Biometric Research Branch performs statistical planning and evaluation of all NCI supported therapeutic clinical trials. The branch performs scientific monitoring for the statistical aspects of the conduct and analysis of trials performed via cooperative agreement or contract. Primary statistical direction is provided by the branch for the conduct of selected national and international studies of therapeutic interventions, prognostic factors, pre-clinical screening and diagnostic imaging. The branch performs evaluations of therapeutic interventions based upon syntheses of results from multiple studies.

The Biometric Research Branch conducts research on experimental designs, biometric methods and biomathematical approaches for the development and efficient evaluation of improved cancer treatments.

PHS 6040 (Rev. 1/84) GPO 904-917

CLINICAL INVESTIGATIONS BRANCH (CIB)
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 disease-oriented contracts, an individual investigator-initiated clinical oncology grant program, a nutrition grant program, the pediatric Phase I contracts, the surgical oncology grant program, a series of Intergroup studies; and scientific administration of the Program Projects grants in clinical cancer treatment.

1.0 Personnel

1. Michael A. Friedman, M.D.--Chief, CIB; Acting Head, Nutrition Section
2. John Y. Killen, Jr., M.D.--Head, Medicine Section, Associate Chief, CIB
3. Richard S. Ungerleider, M.D.--Head, Pediatric Section
4. Frederick Avis, M.D.--Head, Surgery Section
5. Bruce Cheson, M.D.--Senior Investigator, Medicine Section
6. Ann Fields--Secretary to the Chief
7. Carol Tippet--Secretary
8. Jill Johnston--Secretary
9. Helen Bradley--Stay-in-School, Secretary

Dr. Friedman is responsible for the overall administration of the Branch and coordination of its activities with the Cancer Therapy Evaluation Program, the Grants Administration Branch, and the Division of Extramural Activities. Regional Studies Review Committee (CRSRC). He also supervises the Project Officers on clinical contracts, and the Program Directors on grants and cooperative agreements.

Dr. Killen is Associate Chief, CIB and Head of the Medicine Section. He is program director for the Clinical Cooperative Group Program. He is also the Project Officer for the two EMMES Corporation contracts ("Supportive Services for Extramural Clinical Trials" and "Statistical Support for the Gastrointestinal Tumor Study Group"), the Milan, Italy contract "Multimodal Treatment of Primary Breast Carcinoma" and the International Bone Marrow Transplant Registry Contract. He serves as liaison to the EORTC Protocol Review Committee.

Dr. Ungerleider is Head of the Pediatric Section, CIB, and is the Program Director of the P01 and R01 grants for pediatric cancers. He is Program Director for the cooperative groups which are conducting studies of pediatric cancers, and is the Project Officer for the pediatric Phase I contracts. He is program director for the pediatric Program Project Grants.

Dr. Avis is the Head of the Surgery Section. His main responsibility is to promote surgical oncology research on a national level. This is accomplished by heading the program to encourage surgeons to submit R01 and P01 grants under a program announcement, to hold workshops on various timely subjects and promote surgical oncology training programs. He is also the DCT representative to the NCI Nutrition Working Group.

Dr. Cheson is Cancer Expert for the Medicine Section, CIB. He is program director for the P01 Clinical Treatment Grants involving the hematologic malignancies and bone marrow transplantation. He is scientific liaison to

SWOG, CALGB, SECSG, MAOP, Polycythemia Vera Study Group, Leukemia Intergroup, the Lymphoma Pathology Reference Center, as well as the ATLL Project at University of the West Indies, and the RFP for the evaluation of Differentiation Agents in Human Malignancies. Dr. Cheson also functions as CTEP coordinator for extramural activities in AIDS, including the multi-institutional Suramin studies, autologous bone marrow transplantation, interferon therapy in non-Hodgkin's Lymphomas, and hairy cell leukemia. Dr. Cheson served on the planning committee for the Feasibility Study for a National Bone Marrow Transplant Registry.

2.0 Cooperative Agreement Programs

2.1 Cooperative Clinical Trials Group

The present Cooperative Group program was initiated in 1955 by the Cancer Chemotherapy National Service Center, to test new agents from the NCI drug development program. In the early 1970's this grant program was transferred to the Division of Cancer Treatment. During the mid-1970's, NCI initiatives established other disease-specific cooperative clinical trials groups on the advice of advisory bodies which perceived a need unmet at the time by the existing grant-funded program. In 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 this review, and the past history of DCT staff involvement with the cooperative groups, funding for the entire clinical trials program of the Division was converted to the cooperative agreement mechanism in 1982-83. Consolidation of the NCI's cooperative clinical trials programs was completed in 1983 with the transfer of the National Bladder Cancer Treatment Group and the National Prostatic Cancer Treatment group to the DCT.

2.1.1 Listing of the Cooperative Clinical Trials Groups

<u>Multimodality Multidisease Groups</u>

Cancer and Leukemia Group B (CALGB)
Eastern Cooperative Oncology Group (ECOG)
Mid-Atlantic Oncology Program (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)

<u>Multimodality Groups Devoted to a Major Oncologic Area</u>

Children's 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 Rhabdomyosarcoma Study (IRS)
National Surgical Adjuvant Breast and Bowel Project (NSABP)
National Wilms' Tumor Study Group (NWTSG)
Polycythemia Vera Study Group (PVSG)
Radiotherapy Hodgkin's Disease Group (RHDG)
Leukemia Intergroup (LIG)
National Prostatic Cancer Project (NPCP)
National Collaborative Group A (Bladder Cancer)
Brain Tumor Cooperative Group (BTCG)
GI Tumor Study Group (GITSG)
Lung Cancer Study Group (LCSG)

Special Activities Groups

European Organization for Research on Treatment for Cancer (EORTC) Operations and Statistical Office
Lymphoma Pathology Reference Center (LPRC)
Radiologic Physics Center (RPC) (see Radiation Research Program)
Quality Assurance Review Center (QUARC)
Nutrition Oncology Research Cooperative Agreement (NORCA)

2.1.2 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. In 1982 the group elected a new chairman and focussed its scientific scope to the treatment of leukemia, lymphoma, lung and breast cancer.

The Children's Cancer Study Group (CCSG) is a multimodality organization concerned exclusively with pediatric malignancies. They conduct major Phase II and III studies in hematologic and solid tumors, and collect information about the long-term effects of cancer therapy.

The Eastern Cooperative Oncology Group (ECOG), founded in the 1950's, developed and remains committed to multimodal solid tumor studies as well as studies in the hematologic malignancies. This large national group has made major contributions in hematologic breast and gastrointestinal malignancies. It was a pioneer group in the development of a quality control monitoring program and the implementation of biologic response modifiers (BRM) studies.

The Gastrointestinal Tumor Study Group (GITSG) began activity in 1974 as a disease-specific contract-supported research group. Its primary mission has been and continues to be the evaluation

of new surgical adjuvant and combined modality therapies for gastrointestinal malignancy.

The Gynecologic Oncology Group (GOG) coordinates 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 uterine 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 perform research through joint participation of physicians from private practice and academic centers in cooperative clinical effort.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) is a focussed multimodality group. In the past, it concentrated exclusively on primary and adjuvant treatment of breast cancer, but now it is also involved in studies of primary colorectal cancer.

The National Wilms' Tumor Study Group (NWTSG) is an intergroup organization incorporating the pediatric cooperative clinical groups. Their third study (NWTS-3) is primarily concerned with refinement of therapy. NWTS-1 and NWTS-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. They are in the process of developing a successor (NWTS-4) study.

The North Central Cancer Treatment Group (NCCTG), organized in 1980, consists of the Mayo Comprehensive Cancer Center and 10 clinics in the North Central region. The objectives of this Regional Clinical Trials Group is to make 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 and high LET radiation and radiosensitizer studies. They have participated in the direction of the head and neck Intergroup studies and have expertise in biological response modifiers and hyperthermia.

The 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 recently completed a crucial 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 Polycythemia Vera Study Group (PVSG), founded in 1967, had 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 with chlorambucil as compared to radioactive phosphorus 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 diseaseoriented studies exploring more than one modality of treatment using radiation therapy as 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 Southeastern Cancer Study Group (SEG) is a multimodality group involved in studies in leukemia, lymphoma, lung cancer, breast cancer and genitourinary cancers.

The Southwest Oncology Group (SWOG) is a large national group, with a major focus on Phase II and III studies. They have made significant contributions in AML, myeloma, lymphoma, and breast cancer.

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 second study has incorporated special treatment considerations relating to primary site of disease, and prospectively evaluated a new histopathologic grading system. IRS-III has as its objectives improved survival and relapse-free survival rates with the fewest long-term complications.

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 long-term followup.

The Lung Cancer Study Group (LCSG) is composed of member institutions and statistical centers studying surgical, radiation and chemotherapeutic treatments for patients with early lung cancer. Combination studies of chemotherapy with surgery or radiotherapy in patients with localized disease are ongoing.

The National Prostatic Cancer Project (NPCP), converted to a cooperative agreement, is composed of several centers studying chemotherapeutic and hormonal treatments for patients with advanced metastatic disease and evaluates the role of surgery versus radiotherapy in patients with localized disease. Additionally, studies evaluating the role of chemotherapy or hormones as adjuvant for patients with loco-regional disease at high risk risk for dissemination are being conducted. The group is presently involved with a study evaluating flutamide and leuprolide and is responsible for the statistical evaluation of the data.

The National Collaborative Group A (National Bladder Cancer Group) is evaluating the role of intravesical chemotherapy in patients with superficial bladder cancer. Combined modality studies for patients with invasive disease are currently in progress, including one study of adjuvant chemotherapy for patients with invasive disease resected for cure. Additionally, one trial of chemotherapy in patients with metastatic disease is in progress. A large data bank from patients with superficial disease (stages A and 0) has been collected for the past several years and analysis is soon to be completed.

The Brain Tumor Cooperative Group (BTCG) was formerly a contract supported group administered by the Radiation Research Program. It was converted to a cooperative agreement after successful peer review of a grant application in 1983. This group is performing one major Phase III study and one Phase II study in malignant brain tumors. Recent interest has been generated by this group in studying intra-arterial chemotherapy, and refining interstitial radiation approaches.

The following special activities groups provide support services for groups:

The Operations and Statistical Office of the EORTC is partially 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).

The Radiologic Physics Center site visits and reviews the radiation treatment of cooperative groups.

2.1.3 Summary of Accomplishments

Noteworthy accomplishments of the cooperative clinical trials groups are numerous, but selected examples are:

1. CALGB has active, group-wide studies of the lympho proliferative disorders including sophisticated immunological and cytogenetic evaluations, and therapy including interesting new agents such as interferon and deoxycoformycin.

2. SWOG is planning a large Phase III comparison of two of the newer, more aggressive chemotherapy regimens for non-Hodgkin's Lymphoma with CHOP, which has been considered "standard" therapy.

3. In a study of women with advanced breast cancer, MAOP has initiated the first group-wide trial involving response-surface methodology.

4. POG has recently conducted a randomized trial in osteosarcoma which conclusively demonstrated the benefit of postoperative adjuvant chemotherapy over a non-treatment control. This trial addressed a controversial issue whose clarification was considered essential for future studies in osteosarcoma.

5. CCSG has demonstrated that routine histopathologic classification of non-Hodgkin's lymphoma is the best criterion for choice of therapy with non-localized involvement.

6. The National Wilms' Study #3 suggests that tumors designated "favorable histology" can be successfully treated with less intensive regimens. Long-term survival rates of 90% have been demonstrated for patients with non-metastatic favorable histology Wilms' Tumor.

7. CCSG has demonstrated that elevation of serum ferritin levels in neuroblastoma is associated with a significantly poorer prognosis than was normal ferritin and that this correlation is independent of stage and age at diagnosis.

8. The NSABP reported early results from a clinical trial comparing modified radical mastectomy to lesser breast-preserving surgery. The data demonstrate that the lesser surgery is as effective as conventional primary treatment in preventing recurrence. Additional followup is necessary.

9. The GITSG has completed a controlled trial indicating benefit for the use of adjuvant radiotherapy plus chemotherapy compared to surgery alone for patients with resectable rectal cancer.

Intergroup Studies

The importance of participation of multiple cooperative groups in single, high-priority clinical trials is becoming increasingly apparent. Such intergroup studies permit more rapid completion of important trials than is possible by single cooperative groups. During the past year, with considerable involvement by program staff, three new intergroup studies have been initiated. These are:

1. The Intergroup Rhabdomyosarcoma Study Group has developed a prognostic classification system based on cytohistologic subtypes that identifies those patients who require more intensive therapy.

2. Intergroup Prostate Study: This very high priority study will attempt to confirm the impressive, albeit preliminary, results showing that the LHRH agonist, leuprolide, when combined with the antiandrogen, flutamide, yields a very high percentage of prolonged remissions in men with advanced prostatic cancer.

3. Intergroup Colon Adjuvant Trial: This study is designed to replicate an earlier, smaller trial conducted by the NCCTG. Preliminary analysis suggests that patients receiving 5FU plus levamisole following curative surgery for colon cancer may have fewer recurrences than those treated with surgery alone.

Additionally the following studies continue:

1. Intergroup Testicular Study: This is a collaboration among seven cooperative groups 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) versus 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. This study is nearing completion, and full analyses are forthcoming.

2. Intergroup Soft Tissue Sarcoma Study: Patients with primary soft tissue sarcoma (STS) after definitive surgery and/or

radiation therapy are randomized to high dose intermittent adriamycin adjuvant therapy versus a control arm. Most major national and regional groups participate.

3. Intergroup Mesothelioma Study: Patients with limited unilateral thoracic mesothelioma receive combined surgery and radiation therapy and are randomized to receive adjuvant adriamycin versus a control arm. Several major national groups participate.

4. Intergroup Melanoma Study: Patients with intermediate thickness primary melanoma are randomized to have an excision with a 2 cm versus 4 cm margin and to have elective lymph node dissection or not. All cooperative groups plan to participate as well as the NCI of Canada and the Melanoma Clinic, Australia.

5. Head and Neck Intergroup Study: Patients with localized, advanced disease will be treated in a multimodality study.

6. Intergroup Pancreas: Patients with resectable pancreatic cancer will receive post-op therapy in a controlled trial.

3.0 Grant Program

3.1 Program Project Grants (P01)

At the present time there are 31 active clinical program project grants. Program project grants provide research support for broadly based activities that blend preclinical 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.

Among the many interesting and important program project grants are:

1. Frei et al in the Solid Tumor Autologous Marrow Program (STAMP) have presented some of the highest response rates in patients

with advanced cancer treated with high doses of combinations of drugs including multiple alkylating agents.

2. Kersey et al have a program project in pediatric radiation oncology and bone marrow transplantation that is currently investigating the utility of autologous marrow that has been purged of residual leukemia cells by treatment with a "cocktail" of monoclonal antibodies against lymphoblasts. Success in the use of autologous transplantation will widely extend the use of transplantation beyond that currently possible with allogeneic transplant.

3. Simone et al have made many contributions to the therapy of acute lymphoblastic leukemia in children, which include establishing the necessity for CNS therapy, first demonstrating a greater than 50 percent cure rate, and first establishing the clinical relevance of T-cell markers on lymphoblasts. They are currently engaged in attempts to overcome resistance to therapy and to better understand the pathogenesis of childhood leukemias.

5. O'Reilly et al have demonstrated the use of soybean lectin-based fractionation procedure for T-cell depletion in bone marrow transplantation for severe combined immunodeficiency disease.

6. 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 measurements of serum and urinary tumor associated antigens, tumor specific serum antibodies, and circulating immune complexes.

CLINICAL TREATMENT - POIs CATALOG

P.I.	Title
Thomas, E. Donnall	Adult Leukemia Research Center
Clarkson, Bayard D.	Human Hematopoietic Tumors Program Project
Dicke, Karel	Studies in Autologous Bone Marrow Transplantation
Herbst, Arthur L.	UCCRC: Clinical-Laboratory Studies in Gynecologic Cancer
Freireich, Emil J.	Human Leukemia Research Center Program
Storb, Rainer	Aplastic Anemia Center
Schlossman, Stuart	The Biology of Treatment of Human Leukemia and Lymphoma
Rosenberg, Saul A.	Clinical and Laboratory Studies of Malignant Lymphomas
Clarkson, Bayard D.	Cancer Chemotherapy Program Project

P.I.	Title
Laszlo, John	Clinical Cancer Research Program
McGuire, William L.	Medical Oncology Program Project - Therapeutic Research
Carbone, Paul	Experimental Therapy of Solid Tumors
O'Reilly, Richard J.	Transplantation of Lethal Congenital Immunodeficiencies
Moertel, Charles	New Approaches to Treatment of Gastrointestinal Cancer
Santos, George	Bone Marrow Transplantation in Human Disease
Gale, Robert P.	A Program in Bone Marrow Transplantation
Blume, Karl G.	Bone Marrow Transplantation for Hematologic Malignancies
Nathan, David G.	A Program for Investigation of Childhood Tumors
Simone, Joseph V.	Leukemia Program Project Grant
Krivit, William	Pediatric Oncology/Marrow Transplantation Project
Simone, Joseph V.	Studies of Childhood Solid Tumors
Miller, Denis R.	Hematopoietic Malignancies in Children
Morton, Donald	Surgical Immunology and Immunotherapy of Human Cancer
Morton, Donald	New Approaches to Surgical Oncology
Seigler, Hilliard	Diagnosis and New Therapeutic Modalities in Surgical Oncology
Salmon, Sydney	Medical Oncology Program Project
Frei, Emil	Solid Tumor Autologous Marrow Program
Henderson, Edward	Clinical Cancer Research
Bertino, Joseph R.	Clinical Pharmacology and Cancer Chemotherapy
Creaven, Patrick J.	Clinical Pharmacology in Cancer Therapeutics
Wilson, Charles B.	Program for Treatment of Malignant Brain Tumors

3.2 R01 Grant Programs

3.2.1 Clinical Oncology

Description

The purpose of this program is to support research aimed at improved 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 antineoplastic 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 93 grants.

Accomplishments

Many preclinical and clinical studies are worthy of note. A few examples are:

1. Plunkett et al and Capizzi and co-workers are exploring the cellular pharmacology of antitumor agents (especially Ara-C) in human leukemias.

2. Baylin and co-workers are investigating the effects of DMFO in human small-cell lung cancer systems. Particular attention is being paid to polyamine metabolism.

3. Ensminger and colleagues are studying the microcirculation of hepatic tumors and therapeutic intervention with microspheric chemotherapy.

4. 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 tumor metastases to become established.

5. 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.

6. Evans has been investigating the pharmacology of antineoplastic agents in children. Her current studies attempt

to determine whether treatment of acute lymphocytic leukemia in children causes either acute or chronic changes in hepatic clearance of drugs and whether these changes are influenced by the intensity and duration of cancer chemotherapy.

7. Von Hoff et al and Salmon 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.

8. Keefe 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 actions.

3.2.2 Cancer and Nutrition Program

Description

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 anticancer 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. Herber and associates are continuing to examine the efficacy of enteral nutrition 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 to the development of food aversions.

3. Ota et al are conducting studies examining the effect of intravenous nutritional support on the cellular kinetics of rectal carcinoma. Patients are being randomized either to receive immediate surgery or to receive one week of preoperative hyperalimentation. Biopsy specimens collected prior to and during surgery will be analyzed for cellular growth parameters. This study will help to define the role of nutritional support in the cancer patient.

Additionally, supportive care issues are also of interest.

The goals of this effort are 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 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.

Examples are:

1. Dermatologic complications occurring with commonly used anthracyclines, vinca alkaloids, and other effective chemotherapeutic agents can have serious consequences. In a murine model, Dorr et al are exploring the mechanisms of tissue damage, which appear to differ with the various agents. They are evaluating the various treatment approaches which will soon be applied to clinical trials.

2. Nausea and vomiting are frequent and serious toxicities in patients receiving cancer therapy. Ames and co-workers are characterizing the receptors in the central chemoreceptor trigger zone and the interactions of emetogenic chemotherapy agents on these receptors. They will explore the efficacy and effects of commonly used antiemetic drugs in hope of improving the design and approach of antiemetic therapy.

3.2.3 Surgical Oncology

Description

This program was established to stimulate and support surgical oncology research efforts throughout the country and a Program Announcement for R01 and P01 grant applications was issued to encourage these efforts.

Accomplishments

At present there are R01 and P01 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 R01/P01 program announcement three times this year.

In addition one workshop on adjuvant therapy for resection of isolated hepatic metastasis from colorectal cancer has been held and a protocol is being developed. A second workshop dealing with surgical quality control in the cooperative groups is to be held in July 1985 and two other workshops looking at adjuvant therapy to surgery in the treatment of hepatomas and rectal cancers are in the planning stage. The section has been intimately involved in the development of a Surgical Oncology Training Grant to be sponsored by the Division of Cancer Prevention and Control. The Surgery Section is also undergoing a re-evaluation as to its role within NCI and its national responsibilities.

4.0 Contract Programs

4.1 Medicine Section

4.1.1 Istituto Nazionale per lo 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 worldwide attention. Studies testing the value of noncross-resistant drug regimens in the adjuvant setting are currently in progress, as is evaluation of adjuvant therapy in women with negative axillary nodes.

4.1.2 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.

4.1.3 Statistical Support for the GITSG

This contract provides statistical support to the Gastrointestinal Tumor Study Group. As part of the Group's competing renewal application, this effort will be supported in the future under the Cooperative Agreement mechanism.

4.2 Nutrition Section

Five contracts, engaged in a multi-institutional trial studying the effects of aggressive hyperalimentation in small cell lung cancer patients, have recently expired. In a large, prospectively randomized trial which accrued over 100 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 nonmalignant

conditions to evaluate the relationship between diet, activity, and response to therapy by means of direct and indirect calorimetry.

4.3 Pediatric Section

Phase I Clinical Trials and Pharmacokinetics Studies in Children

This contract is for studies in childhood malignancy: to define the acute toxicities of new anticancer agents, to define the dose of each agent which can be safely given in subsequent trials of efficacy, to provide information on the pharmacologic characteristics of selected antitumor agents, and to investigate age-related differences in these pharmacologic characteristics.

4.4 Extramural Clinical Trials Office (ECTO) - EMMES

This contract provides operations and administrative support for a number of CTEP supported extramural research efforts. 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. The contractor also provides analytical support to CTEP in evaluating data obtained from extramural clinical research resources.

4.5 NCI-Pan American Health Organization: Collaborative Cancer Treatment Research Program (PAHO:CCTRP)

The collaboration between U.S. and Latin American investigators in the development of clinical studies continues to be the major goal of this program. An extensive reevaluation of goals has been done, resulting in: 1) concentration primarily in Phase II trials in diseases prevalent in the area such as head and neck, gastric, lung and cervical cancers; 2) design of master Phase II protocols in the disease 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 effort 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 versus 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 intraarterial 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. Two multinational Phase II trials in gastric cancer have been completed, and an additional two are soon to be activated. A multinational randomized Phase II study with the platinum analogs (CDDP and CBDCA) in metastatic cervical cancer are in progress.

5.0 Miscellaneous

5.1 Clinical Trials Review

As part of an ongoing effort to evaluate the quality of science, efficiency and clinical impact of the Cooperative Agreement mechanism, an orderly series of reviews of disease-specific topics are being held. Consultative groups of internists, radiation therapists, surgeons, and statisticians from the extramural and intramural community are reviewing the last seven years of Cooperative Group efforts - disease by disease. Thus far, testicular cancer and non-small cell lung cancer have been reviewed. The emphasis is not on the performance of any particular cooperative entity, but rather on the mechanism as a whole.

5.2 Suramin Programs for HTLV Patients

Most recently, Dr. Cheson has coordinated a multi-center study of the reverse transcriptase inhibitor Suramin in patients with AIDS, ARC, and Kaposi's Sarcoma. These studies entail virologic, immunologic and clinical endpoints of interest.

6.0 Staff Publications

Cheson, B.D.: Clinical Utility of Body Fluid Analyses. Symposium on Body Fluid Analysis. Ed.: G.B. Schumann. Philadelphia, PA, W.B. Saunders Co., 1985. pp. 195-208.

Cumberlin, R., DeMoss, E., Lassus, M., Friedman, M.: Isolation Perfusion for Malignant Melanoma of the Extremity: An RDB and CIB Review. J. of Clin. Onc., in press.

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Hoffman, F.: Micronutrient requirements of cancer patients. Cancer Supplement, Vol. 55, No. 1, pp. 295-300, 1985.

Holcenberg, J.S., Tutsch, K., Earhart, R. Ungerleider, R.S., Kamen, B.A. Pratt, C.B., Gribble, T.J., and Glaubiger, D.L.: Phase I Study of ICRF-187 in Pediatric Cancer Patients: a Rationale. In: Pharmacology in Pediatric Oncology. Eds.: Massimo, L., Cornaglia-Ferraris, P., and Poplack, D.G. Boston, Martinus Nijoff, in press.

Killen, J.Y., Ellenberg, S.S.: Adjuvant chemotherapy and immunotherapy of gastrointestinal cancer. In: Frontiers in Gastrointestinal Cancer. Eds.: B. Levin and R. Riddel. New York, Elsevier, 1984, pp. 261-272.

Kishter, S., Hoffman, F., Pizzo, P.: Production of and response to interleukin-2 by cultured T-cells: effects of lithium chloride and other putative modulators. J. of Biolog. Resp. Mod., Vol. 4, pp. 185-194, 1985.

Marsoni, S. Ungerleider, R.S., Hurson, S.B., Simon, R.G., and Hammershaimb, L.D.: Tolerance to Antineoplastic Agents in Children and Adults. Cancer Treatment Reports, in press.

Meguid, M. Wahba, A., Hoffman, F.: Fourth annual nutrition symposium on current concepts in nutrition management of the patient with cancer. Vol. 55, No. 1, pp. 217-310, 1985.

Ungerleider, R.S., Pratt, C.B., Vietti, T.J., Holcenberg, J.S., Kamen, B.A., Glaubiger, D.L., and Cohen L.F.: Phase I Trial of Mitoxantrone in Children. Cancer Treatment Reports 69:403, 1985.

Ungerleider, R.S. and Marsoni, S.: Phase I and II Trials in Pediatric Cancer patients: a Rationale. In: Pharmacology in Pediatric Oncology. Eds.: Massimo, L., Cornaglia-Ferraris, P., and Poplack, D.G. Boston, Martinus Nijoff, in press.

Wittes, R.E., and Killen, J.Y., Jr.: Problems in the Therapy of AIDS-Associated Malignancies. In: Acquired Immune Deficiency Syndrome. Eds.: Selikof, I.J., Teirstein, A.S., and Hirschman, S.Z., New York, New Yor, The New York Academy of Sciences, 454-460, 1984.

7.0 Presentations

John Y. Killen, M.D.

1. Cancer of the Pancreas. At: Seminars in Oncology. Howard University Cancer Center, Washington, DC, August 2, 1984.

2. Combination Chemotherapy Regimens in Advanced Gastric Cancer. American Society of Clinical Oncology, Houston, TX, May 21, 1985.

Michael A. Friedman, M.D.

1. Systemic Therapies for Patients with Liver Tumors - Prospects for the Future. At: Conference on Therapeutic Stratgies in Primary and Metastatic Liver Cancer. Heidelberg Germany, September 19, 1984.

2. Biochemical modulation in the setting of regional chemotherapy. At: American Society for Clinical Pharmacology and Therapeutics Section of Hematologic and Neoplastic Diseases. San Antonio, TX, March 30, 1985.

3. Adjuvant Treatments. At: Colon Cancer Update - American Cancer Society. Alexandria, VA, April 17, 1985.

Richard S. Ungerleider, M.D.

1. Phase I Trials in Children. At: U.S.-Japan Agreement. Honolulu, HA, February 25, 1985.

2. Phase I and Phase II Trials in Pediatrics. At: Advanced Course on the Role of Pharmacology in Pediatric Oncology: Principles, New Drugs and Novel Therapeutic Approaches. Erice, Italy, May 5, 1985.

8.0 Conferences, Workshops, Seminars

Frederick P. Avis, M.D.

1. Workshop for the Design and Implementation of Adjuvant Treatments Following Resection of Hepatic Metastases for Colorectal Cancer. Bethesda, MD, May 9-10, 1985.

Bruce D. Cheson, M.D.

1. Clinical Trials with Suramin in Patients with AIDS. Bethesda, MD, October 10, 1984.

2. Working Session on Clinical Trials Using High Dose Chemotherapy and Autologous Bone Marrow Transplantation. Bethesda, MD, April 10, 1985.

Richard S. Ungerleider, M.D.

1. Workshop on the Childhood Histiocytoses: Concepts and Controversies. Philadelphia, PA, May 16-17, 1985.

INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch has the mission of developing new investigational drugs by sponsoring clinical trials to evaluate their pharmacology, toxicities, and efficacy. It does this by: (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) developing and implementing a plan for Phase II trials in specific tumor types, and monitoring the results of these clinical trials, (4) developing and implementing in collaboration with the Clinical Investigations Branch of CTEP Phase III trials in selected areas of promising activity observed in Phase II, (5) meeting FDA regulatory requirements for all active INDs, (6) regulating the distribution of investigational new drugs, and (7) reviewing and assuring the quality of clinical trials or its sponsorship as well as its compliance with regulatory requirements.

The Investigational Drug Branch is now divided into five sections. Two medical sections, one for the cytotoxic agents and one for the biologic response modifiers which are concerned with the clinical aspects of the drug development process; the Drug Regulatory Affairs Section which provides a constructive interaction with the FDA; the Drug Management and Authorization Section which regulates the distribution of investigational new drugs to all NCI sponsored investigators; and finally a Quality Assurance and Compliance Section which 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.

Highlights of the year include:

(1) Introduction of 15 New Drugs into Phase I Trial: Of particular interest are Phase I trials conducted with Flavone Acetic Acid, an agent with broad solid tumor activity in preclinical testing, and Suramin, the first agent introduced into clinical trial as an antiviral agent for the treatment of AIDS. Among the biologics there were three monoclonal antibodies tested.

(2) Drugs Entering Phase II: The following drugs entered Phase II trials during the year: Pibenzimol, Trimetrexate, Taxol, Caracemide, Acodazole, and SR-2508.

(3) Creation of a Biologics Evaluation Section: During this year two physicians were recruited to begin the development of a program of Phase II and III trials with biologic agents. Its initial activity was to undertake a comprehensive review of the current status of the interferons. As a result of this review, critical studies were identified for new research in chronic myelogenous leukemia, hairy cell leukemia, and non-Hodgkin's lymphoma. In addition the staff is attempting to organize a definitive trial of the role of BCG for the prevention of recurrence of superficial bladder cancer.

(4) Important Clinical Trials Initiated: During the past year several clinical trials are worthy of special note. A Phase I program with Suramin was the first widespread test of an agent specifically directed against the HTLV-III virus. Promising Phase II results with Ifosphamide in metastatic sarcoma have lead to the initiation of a Phase III trial which will determine whether it will contribute to the standard therapy of that disease.

(5) Expanded Efforts in Development of Clinical Trials Based on Biochemical Modulation: During this year the staff developed an RFA to stimulate interest in conducting clinical Phase I and II trials which test whether combinations of drugs based on laboratory demonstration of interaction at the biochemical pathway level produce similar perturbations in man, and result in improved therapeutic results.

(6) Scientific Reviews:

- A major analysis of the results of fifteen years of Phase II trials sponsored by DCT has been completed during this year. Initiated more than two years ago this review encompassed abstracting more than 2500 Phase II trials. It is expected that this review will be published during FY'86.

- An analysis of the correlation between the activity of new drugs in murine systems and the observed activity in human trials was completed during this year. This analysis served in part as the foundation for major changes in the procedures for screening for new drugs of the Developmental Therapeutics Program.

- In-depth reviews were published by the staff for many of the drugs under IDB sponsorship including Etoposide, Carboplatin, Hexamethylmelamine, Echinomycin, Dichloromethotrexate, Trimetrexate, Spiromustine, N-Methylformamide, Tiazofurin, and Deoxycoformycin.

(7) Scientific Sessions: Meetings with the Phase I investigators were held three times during the last year. At each a half-day seminar featuring some topic of preclinical research with relevance to clinical drug development was held. Included in these were seminars on PolyADP Ribosylation, Differentiating Agents, and Polyamines.

(8) Drug Development Seminars: During the past year a seminar program was initiated in which IDB staff critically review the current status of an investigational drug. These seminars are intended to serve as the basis for the creation of a drug development plan for each drug.

(9) Major Changes in Drug Status:

- FDA approved Leuprolide during 1985 for the treatment of prostatic cancer.

- IND closures: The INDs for Maytansine and Trifluorothymidine (F3TDR) were closed.

(10) Clinical Trial Monitoring and Organization:

a. Investigator Handbook - An investigator handbook was developed which is a comprehensive description of the policies and procedures of the CTEP for the conduct of clinical trials using its investigational drugs. As a result, for the first time investigators now have all information required to develop, conduct, and report a clinical trial in one place.

b. Drug Development Contracts - The drug development contracts underwent a major restructuring. The Phase I and Phase II/III contracts were issued as one RFP. The Phase I contracts now include substantially more pharmacokinetic and pharmacology studies.

c. Data Management and Monitoring Capabilities - A standard DCT case report form was developed during the year. It is expected that this will serve as the standard format for reporting of data to CTEP under the sponsorship of contracts and cancer center studies. In addition a study summary form was developed which when implemented will provide CTEP with a uniform data set for all of its Phase I and Phase II trials of investigational anticancer drugs.

d. Clinical Trials Monitoring Service was significantly upgraded in its data handling capabilities. The mainframe computer data management system for Phase I and Phase II data can now accept data both by direct transfer from microcomputer as well as by magnetic tape input. The microcomputer data entry is accomplished by a software package permitting entry of data for Phase I and Phase II trials. It is expected that this will be principal method of data submission by the Phase I contractors.

e. Toxicity Criteria - Meetings were initiated with representatives of the cooperative groups to develop common toxicity criteria for trials of investigational drugs.

(11) Drug Management and Distribution: During the past year the use of the investigational drugs for compassionate non-protocol use was carefully scrutinized. As a result investigational drugs are now distributed for non-protocol use less frequently, and only in specific clinical circumstances.

(12) Drug Costs: During the past year a major reduction in drug costs was achieved. This was accomplished through several routes including discontinuing several clinical trials which were currently deemed of little scientific value, encouraging significant cost sharing on the part of the pharmaceutical industry, and tighter management by the Drug Management and Authorization Section of drug distribution. One important aspect was a protocol cap, which permits DCT to better manage its drug distribution.

BIOLOGICS EVALUATION SECTION

Evaluation of BCG in Bladder Cancer. Two cooperative group trials evaluating the use of BCG in superficial bladder cancer are currently in the process of being designed. A conference was held to identify the issues unique to the design of trials in superficial bladder cancer and to develop standards for the conduct of these trials.

Evaluation of Interferon in Chronic Myelogenous Leukemia. A multicenter study has been arranged to evaluate the effect of Interferon in CML when compared with standard therapy. In a separate trial the effect of intensive chemotherapy followed by Interferon will be compared to the use of Interferon alone.

Establishment of a Working Group to Evaluate Antiviral Compounds in Patients with AIDS. Seven extramural centers have been selected to investigate new agents which might be active in the treatment of AIDS. Each center was selected for their ability to monitor virus isolation, drug pharmacokinetics, and immunologic function of the patients. The institutions were University of California at San Francisco, University of California at Los Angeles, University of Southern California, Beth Israel Hospital in New York, and Walter Reed Medical Center. These centers will conduct the initial testing of compounds and will provide data which permit rational design of larger scale trials.

DEVELOPMENTAL CHEMOTHERAPY SECTION

Phase I Studies. Five investigational drugs sponsored by the DCT were newly introducted into Phase I clinical trials.

Sodium Thiosulfate is a thiol-containing compound that has been shown to protect against cis platinum-nephrotoxicity in rats and humans. It appears to act selectively as a protective agent for the kidneys. It is concentrated in the renal tubules and reacts directly with cisplatin forming a cisplatin-complex which is neither nephrotoxic nor cytotoxic.

Human pharmacokinetic studies of i.v. sodium thiosulfate have determined its elimination to be best described by a first order, one-compartment kinetic model with a plasma elimination half-life mean of 80 + 38 minutes. Four Phase I trials using three different routes of cisplatin administration (intraperitoneal, intravenous and intracavitary) were conducted to determine the clinical efficacy of sodium thiosulfate in preventing cisplatin induced nephrotoxicity. In the intraperitoneal studies, it was determined that cisplatin could be escalated to 270 mg/m^2 with intravenous sodium thiosulfate administration without producing significant nephrotoxicity as dose-limiting toxicity.

The NCI intends to sponsor intraperitoneal cisplatin-thiosulfate studies in CALGB and SWOG in ovarian cancer.

Flavone Acetic Acid is a synthetic flavone derviative which demonstrated DN-2 activity against the s.c. implanted colon 38 tumor following either i.p. or i.v. administration. Activity was also observed against the i.p. implanted L1210 leukemia and B16 melanoma. The mechanism of action is unknown. In in vitro studies in P388 with the parent compound, the synthesis of macromolecules was not significantly perturbed. It was suggested that the compound may interfere with Na-dependent ATPase.

Merbarone is a novel synthetic compound which demonstrated significant activity against the i.p. implanted L1210 leukemia and B16 melanoma. Activity was observed against the s.c. implanted L1210 leukemia and the i.p. implanted M5076 sarcoma.

Deoxyspergualin is a novel antibiotic from the Microbial Chemistry Research Foundation in Japan which passed Decision Netowork based on its activity against the i.p. and s.c. implanted L1210 leukemia. Moderate activity was demonstrated against the i.p. implanted P388 leukemia, while marginal activity was seen against the B16 melanoma. The mechanism of action of this polyamine derivative is not known. Ornithine dexarboxylase was not inhibited at 1.5 mg/ml, and S-adenosylmethionine decarboxylase was only weakly inhibited (IC50=1 mg/ml). Nucleic acid and protein synthesis were not inhibited in cultured L1210 cells at concentrations as high as 100 g/ml, and the drug had no effect on binding of colchicine or vincristine to tubulin.

Pyrazole is a potent inhibitor of alcohol dehydrogenase and has been used for many years in studies related to the metabolism of ethanol In rats, pyrazole significantly depresses serum thyroxine and triiodothyronine levels with a resulting increase in TSH. Based on these findings the suggestion was made that pyrazole in small doses might be suitable for inducing chemical thyroidectomy or suppressing thyroid tumors.

Pyrazole was originally selected for clinical trials on the basis of its activity against Carcinoma 755.

Two Phase I studies have been reported in cancer patients treated with pyrazole. Twenty-nine patients were given i.v. pyrazole at doses ranging from 3 to 24 mg/kg/day for five days. Hepatic toxicity was most prevalent at all dose levels. At higher doses severe nausea and vomiting with anemia and renal toxicity were observed. No responses were noted.

Because of the demonstrated selective destructive action of pyrazole against normal thyroid tissue in animals it has been considered for a Phase II study in patients with thyroid carcinoma.

In addition to the Phase I drugs just mentioned Phase I trials were continued from last year on the following drugs:

1. Tiazofurin. Based on the findings of Jayaram and Weber that a metabolite of Tiazofurin, TAD, inhibited IMP Dehydrogenese in vitro for three days, a new new Phase I trial testing a twice weekly schedule for three weeks was initiated. The intent is to examine the effect of continuous inhibition of IMPD in man for a three week period. This was accompanied by both pharmacokinetics of tiazofurin and measurement of IMPD inhibition in the marrow.

2. I.V. 6-MP. These trials were completed by the Mayo Clinic.

3. Spiromustine. The CNS toxicities of Spiromustine were found to be partially alleviated by physostigmine. Phase I trials are closing on this compound and tumor specific (glioma) Phase II trials are being initiated.

4. Echinomycin.

5. Fludarabine Phosphate. Fludarabine resulted in a high complete remission rate in patients with AML at doses greater than 100 mg/m^2, but was very often associated with neurotoxicity above that level. Cortical blindness and dementia are the dose limiting toxicity in the leukemia trials. Work is progressing with doses of 60-90 mg/m^2 in more prolonged schedules of administration in an attempt to improve therapeutic index in acute leukemia. A large number of Phase II trials in various solid tumors are being conducted at doses approximating 25 mg/m^2.

6. Homoharringtonine. Phase I trials are completed both in solid tumors and leukemia. Phase II trials in leukemia and solid tumors are proceeding.

7. Deoxydoxorubicin. In broad Phase II testing.

8. N-Methylformamide. Oral Phase I testing with in-depth pharmacokinetics are now being conducted, since significant hepatotoxicity was seen at the higher dose ranges of an initial oral Phase I trial.

9. CBDCA. Is now being tested in approximately 60 Phase II trials covering the whole range of solid tumors and leukemia (adult and pediatric).

10. Trimetrexate. A broad range of Phase II trials are underway. Since this drug shows considerable schedule dependency in animal models, Phase III randomized trials of this drug will assess whether the preclinical scheduling screen is translatable into man. These studies are being conducted in association with biochemical measurements of DHFR inhibition in the bone marrow of patients.

11. Pibenzimol is still in Phase I testing. Of interest is the significant pancreatic injury in the Phase I trial. This led to further animal tumor model testing in association with DTP, Battelle Laboratories, and Ohio State University before the re-initiation of Phase I trials.

12. HMBA is a polar planar compound that selectively differentiates Friend erythroleukemia and HL60 cells in vitro at non-cytotoxic doses. This drug is now well into Phase I trials, and at 19.2 g/m^2 given as a continuous infusion over 5 days, plasma levels over 1 mmolar are obtained. This was the concentration necessary to induce differentiation in the in vitro models. Since the extended differentiation is dose-dependent, Phase I trials are continuing in order to escalate the dose further.

13. Didemnin B is now completing Phase I clinical testing and is about to enter a broad range of Phase II solid tumors and leukemia.

Phase II clinical trials are continuing on 35 cytotoxic drugs in the Section.

Extensive plans have been drawn up for the two platinum analogs, CBDCA and CHIP in ovary, small cell lung, and head and neck tumors so to specifically define the role of these drugs in front line therapy, with defined endpoints of either increased efficacy or reduced nephrotoxicity. Randomized scheduling trials on trimetrexate in head and neck cancer and possibly breast cancer are being negotiated at the present time. Ifosphamide has been shown to have significant activity in adult soft tissue sarcoma, and three randomized Phase III trials (intergroup sarcoma, NCI-C, and ECOG) are currently being developed to define its role in combination with Adriamycin plus + DTIC in front line therapy. The role of Homoharringtonine in combination therapy (especially with VP-16) is still to be determined in acute myelocytic leukemia. Similarly the role of deoxycoformycin + Interferon is being determined in hairy cell leukemia. A randomized Phase III trial of non-cross resistant regimens including the M.D. Anderson MIME regimen is now being negotiated between M.D. Anderson and Memorial Hospital.

Case Report Forms. A uniform format of reporting Phase I and Phase II data has been developed which identifies critical data elements necessary for analysis of these trials.

A remote data entry system has been initiated so that this data may be transmitted electronically and collected by CTMS.

DRUG REGULATORY AFFAIRS SECTION

IND Submissions.

For the FY'85, a Notice of Claimed Investigational Exemption for a New Drug (IND) was submitted to the Office of Drug Research and Review, Food and Drug Administration (FDA), for each of the following five compounds:

Drug	NSC Number
Sodium Thiosulfate	NSC-45624
Flavone Acetic Acid	NSC-347512
Merbarone	NSC-336628
Deoxyspergualin	NSC-356894
Pyrazole	NSC-45410

The INDs for the following drugs were discontinued due to a lack of clinical activity:

Drug	NSC Number
Maytansine	NSC-153858
Trifluorothymidine (F3TDR)	NSC-75520

INDs were submitted to the Office of Biologic Research and Review, FDA, for the following biological response modifier agents:

Monoclonal Antibody to TAC	NSC-377524
Monoclonal Antibody to TAC Conjugated to Pseudomonas exotoxin	NSC-600665

Cross-filed INDs were submitted to FDA for the following agents:

Difluoromethylornithine	NSC-337250
Flutamide	NSC-147834
Leuprolide	NSC-377526
Recombinant IL-2	NSC-600664
Recombinant Gamma Interferon	NSC-600662
Monoclonal Antibody to Human Colorectal Cell Lines	NSC-377963
Natural IL-2	NSC-600663
A5094	NSC Number to be Assigned

The Drug Regulatory Affairs Section currently maintains 122 active INDs for both cytotoxic and biologic compounds.

Adverse Drug Reaction Reporting

The Section is responsible for the reporting adverse drug reactions to FDA. During CY'84 151 adverse drug reactions were reported to FDA.

A standard format for collecting data on ADR's has been established, and will permit the first comprehensive analysis of ADR's in cancer drug development.

QUALITY ASSURANCE AND COMPLIANCE SECTION

In 1982, the Investigational Drug Branch initiated a site visiting program to monitor clinical trials performed by the clinical cooperative groups and cancer centers. On-site monitoring of Phase I trials had been implemented in 1979.

In the case of the cooperative groups, NCI has delegated the responsibility for organizing and conducting the monitoring program to each group. All 19 cooperative groups supported through the Cancer Therapy Evaluation Program now have active monitoring programs. Since 1982, a total of 475 member institutions and 465 affiliate institutions have been site visited; during the current year 114 members and 66 affiliates were monitored. In addition, 45 CCOPs have been site visited.

The Quality Assurance and Compliance Section continues to co-site visit with the cooperative groups in 10-20% of the scheduled visits to assure the adequacy of the audit procedures. In addition, the cooperative groups submit a report on each monitoring site visit to the Section for review.

The Quality Assurance and Compliance Section directly oversees the monitoring of Phase I and cancer center studies. Phase I studies are monitored three times per year. Since 1982, 43 cancer centers site visits (involving 33 institutions) have been accomplished. Sixteen were carried out in the current year.

In October 1984, a symposium involving representatives from the cooperative groups, cancer centers, and the pharmaceutical industry was sponsored by the Section to explore aspects of clinical trials methodology and quality assurance. The proceedings published from this symposium will include a manuscript documenting the results of the first three years of NCI's monitoring program and its effect on the quality of clinical trials supported by the NCI.

DRUG MANAGEMENT AND AUTHORIZATION SECTION

Drug Accountability. The drug accountability system, implemented in January 1983, has continued to function well. All investigational drugs must be ordered and dispensed to patients by protocol and documented on a drug accountability form. Drug accountability has proven to be an essential addition to the site visit monitoring as conducted by the Quality Assurance and Compliance Section. The Drug Accountability Records Form and instructions were approved by the Office of Management and Budget as an official form. In addition, the form is being accepted by several drug companies.

Drug Distribution and Computer Modifications. During the past year, the Drug Distribution Protocol Monitoring System has been enhanced by the development of standardized computer queries designed to report drug distribution by protocols and investigators at particular institutions. This serves as a guide

for conducting site visits. Seventy-nine special queries have been provided for use in-house and to the FDA.

CCOP Investigators. Many CCOP investigators have been registered to receive investigational drugs.

Site Visit to Investigators. The drug accountability system has been site visited in several institutions, usually in conjunction with the medical site visit. The drug accountability system has provided a new dimension in the tracking of investigational drugs.

Special Exception (Compassionate) Use of IND Drugs. The administrative management of the Special Exception protocol drug distribution system was transferred to this Section during the past year. A written set of policies and procedures governing the use of Special Exception drugs has been established and implemented which has improved the protocol management compliance.

These guidelines have provided the necessary information for physicians to improve their compliance in returning the investigational protocol for submission with the FDA. A savings of $57,216 has been realized with Special Exception drug during the first two quarters of FY'85.

Investigator Annual Registrations. Policies and procedures have been improved with less paperwork during the past year for annual registration of investigators. The compliance is presently 100%.

Drug Cost Reduction. A number of procedures, i.e. reduction in the use of commercially available drugs, closer scrutiny of orders for obtaining Group C and Special Exception drugs, etc., have been implemented. These improved procedures for handling Group C and Special Exception drugs have resulted in decreased drug cost, $51,216 and $84,039, respectively. These and other procedures have significantly reduced the total drug budget. The savings for FY'84 was $1,402,467 and for the 1st and 2nd quarter FY'85 $224,548 as compared to the immediate prior period of time.

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 on Group C Drugs Request	Total Number for Special Exceptions
11,811	19,138	1,905	2,903

Special attention is given to making THC, a Schedule I controlled substance, available for patient treatment. In 1984, the following statistics apply to the THC antiemetic program:

THC-CY'84

No. of Hospital Pharmacies Participating		629
No. of Physicians Registered to Prescribe THC		2,603
No. of Capsules Distributed	2.5 mg.	42,600
	5.0 mg.	162,025
Approximate No. of Patients Receiving THC	2.5 mg.	1,419*
	5.0 mg.	5,205*
No. of State Sponsored Pharmacies Receiving THC		67
No. Individual Investigators Receiving THC		2

* If patients received THC in more than one calendar-quarter, they are counted more than once. If patients received 2.5 and 5.0 mg. capsules, they are counted more than once.

Management techniques have been applied to improve the inventory control for THC, thus reducing pharmacy investigators as well as reducing overall THC costs.

A computer alert system has been established and implemented to assure that investigators:

1) Return Special Exception protocols for FDA submission,

2) Return FDA Form-1573 to IDB,

3) Comply with quality assurance standards of IDB, and

4) Maintain investigational drug accountability records in accordance with Sponsor/FDA requirements. This mechanism has been very successful for the NCI to be assured FDA requirements are maintained.

A protocol cap has been established to alert the CTE Program about the quantity of drug distributed for each protocol.

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SUMMARY REPORT
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
OCTOBER 1, 1984 - SEPTEMBER 30, 1985

I. INTRODUCTION

The Radiation Research Program (RRP) was established in 1982 within the Division of Cancer Treatment. The three Branches which originally comprised the Program were the Diagnostic Imaging Research Branch (DIRB), the Radiotherapy Development Branch (RDB), and the Low-Level Radiation Effects Branch (LLREB). In FY 1984, it was determined that the mission of the LLREB better coincided with that of the Division of Cancer Etiology, and the Branch was transferred.

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. An additional major mission of the DIRB is to establish effective working relationships with the other Divisions within NCI and also with other NIH institutes which substantially fund research related to diagnostic imaging. The RRP also provides a focal point on radiation research within NIH for extramural investigators both nationally and internationally.

II. PERSONNEL

A. Staffing

1. Office of the Associate Director

Francis F. Ruzicka, Jr., M.D., Acting Associate Director
Bonnie Jenkins, Secretary to Associate Director
Jan Johnson, Technical Assistant
Theresa Phillips, Clerk-Typist

2. Administrative Office

Barbara Vermillion, Administrative Officer
Barbara Levin, Budget Assistant

3. Diagnostic Imaging Research Branch

Francis Ruzicka, M.D., Chief
Matti Al-Aish, Ph.D., Deputy Chief
Roger Powell, Program Director
Ann Malner, Branch Secretary

4. Radiotherapy Development Branch

Francis Mahoney, Ph.D., Acting Chief
Robert Morton, M.S., Program Director
Sandra Zink, Ph.D., Cancer Expert
Thomas Strike, Ph.D., Program Director
Maureen Volz, Statistical Assistant
Anne Gentilcore, Branch Secretary

B. Recruitments

Associate Director
Chief, Radiotherapy Development Branch
Vacant, Secretary, Office of Associate Director

III. MAJOR ACTIVITIES

The major activities of the RRP have been devoted to managing extramural radiation research activities in DCT and NCI and to developing new initiatives. To further these goals long-range plans have been developed for diagnostic imaging, radiation physics, radiation therapy and radiobiology.

A long-range plan for diagnostic imaging research was developed in 1983 under the direction of Dr. Gabriel Wilson, Chairman, Department of Radiology, University of California, Los Angeles, during his year with the Radiation Research Program. This plan was published in Investigative Radiology in mid-1984, and contains reports of Task Forces dealing with a broad spectrum of imaging of the various organs and body systems, and the new advanced technologies with their related basic physics and engineering fields. During the current year, a workshop on "Directions in Research" (see below) has further defined the more immediate and currently pertinent research areas.

A long-range plan for radiation oncology, biology, and physics was developed in 1983 under the direction of Dr. Glenn Sheline, Vice-Chairman of Radiology, University of California, San Francisco, during his year with the Radiation Research Program. The plan was published in Cancer Treatment Symposia in early 1984. It is from this plan that several new initiatives have originated during the current year.

WORKSHOPS

Ideas for research/initiatives come from a variety of sources. One of the more productive sources is the workshop designed for this purpose.

DIAGNOSTIC IMAGING RESEARCH BRANCH

Three workshops and one mini-workshop were carried out during this fiscal year by the DIRB. These were respectively entitled, "Future Perspectives for Magnetic Resonance Imaging and Spectroscopy" held on October 11-12, 1984; "Perception of Radiologic Information" on April 15-16, 1985; "Directions in Research in Diagnostic Imaging and Nuclear Medicine" on May 2-3, 1985, and "Improvement and Development of Radiopharmaceuticals for Employment with Single Photon Emission Computed Tomography (SPECT)" on May 17, 1985.

"Future Perspectives for Magnetic Resonance Imaging and Spectroscopy"
Magnetic Resonance Imaging (MRI) is the most recent of a series of remarkable technologies that have developed in diagnostic imaging. One can produce not only head and body images similar to those seen with Computed Tomography but by means of Magnetic Resonance Spectroscopy, it is possible to obtain data on a cellular level. By identifying certain products of metabolism by means of phosphorus spectroscopy, researchers have been able to differentiate human small cell from non-small cell carcinoma of the lung and recognize other malignancies. Resulting from this workshop is a program announcement entitled, "Combined In Vivo Application of Multi-Nuclear Magnetic Resonance Imaging and Spectroscopy" that invites applications dealing with a broad spectrum of multinuclear imaging correlated with spectroscopic evaluation with a major thrust toward anatomical delineation (MRI) and correlated tissue specific diagnosis (MRS).

"Perception of Radiologic Information"
The perception of medical images and how one thinks about what one sees were the main topics of this workshop. Perception and recognition have been problems since the inception of x-ray imaging. Various studies of errors of interpretation of images have been made in the past. An error rate of up to 30% has been reported. The newer technologies of the present day have further complicated the issue and new studies are needed to evaluate the newer types of image acquisition and display. It was concluded that research directed toward developing models of human perception and decision-making should be continued. Much work is needed also in image digitization and in improving image display systems.

"Directions in Research in Diagnostic Imaging and Nuclear Medicine"
The goal of this workshop was to revisit the comprehensive report, "Plan for Diagnostic Imaging Research-Report of Task Force", which was published in Investigative Radiology* and to update and focus in on initiatives most suitable for research in the immediate future. The major recommendations resulting from this meeting propose the application of imaging modalities toward the goal of tissue diagnosis. Areas where work has already begun and which

*Volume 19/3 Supplement, May/June 1984

are most promising are combined magnetic resonance imaging and spectroscopy, the employment of monoclonal antibodies, and tissue characterization with ultrasound.

"Improvement and Development of Radiopharmaceuticals for Employment with SPECT", This workshop was held to update a previous workshop of FY 1984 on the subject of the development of new radiopharmaceuticals intended for use with SPECT in the investigation of metabolic and physiologic processes. Tc-99m and I-123 are the most promising nuclides for this purpose at this time.

RADIOTHERAPY DEVELOPMENT BRANCH

During FY 1985, the RDB sponsored four workshops on topics of potential future solicited research. These were: (1) Planning Workshop for Future Patterns of Care Studies, (2) Normal Tissue Tolerance to Combined Modality Therapy Using Radiation and Chemotherapy, (3) Verification of Patient Treatment Fields in Radiation Therapy, and (4) Phase III Clinical Trials Using Photodynamic Therapy.

"Future Patterns of Care Studies"
On April 15-16, 1985 a planning workshop was held in Philadelphia, on Future Patterns of Care Studies. The original Patterns of Care in Radiation Therapy Study was extremely successful. The results of this workshop will be similarly used to help plan a new study to extend and expand the original work.

"Normal Tissue Tolerance to Combined Modality Therapy Using Radiation and Chemotherapy"
A workshop is planned for September 3-5, 1985 to discuss what is known about Normal Tissue Tolerance to Combined Modality Therapy Using Radiation and Chemotherapy and what research still needs to be done to improve understanding and to reduce patient morbidity.

"Verification of Patient Treatment Fields"
Geometric accuracy and reproducibility of patient treatment fields in radiation therapy will be the subject of a workshop planned for September 12-13, 1985. This will be a presentation and discussion of what is currently being done to assure that the radiation is delivered to the volume of the patient for which the radiation oncologist has prescribed treatment and to discuss research needed to improve the accuracy and reproducibility of treatment delivery.

"Photodynamic Therapy"
The objective of this workshop held on September 28, 1984, was to focus on the problem areas in Photodynamic Therapy (PDT). During the course of this session, the participants discussed and prioritized problem areas which included instrumentation, light delivery systems, light dosimetry, drugs, and basic photochemical and photobiological actions of both the drug (porphyrins) and light at the molecular, cellular and tissue levels. It was the feeling of this group of experts that despite the need for additional work in various basic aspects of PDT, randomized Phase III trials should be started in limited anatomical sites.

IV. RADIATION RESEARCH PROGRAM RESEARCH GRANT AND CONTRACT SUPPORT

FY85 BUDGET
(Dollars in Thousands)

	Contracts Number/Amount	Grants Number/Amount
Diagnostic Imaging Research Branch	5 / 1,168	98 / 16,192
Radiotherapy Development Branch	26 / 7,394	198 / 40,328
TOTAL RRP	31 / 8,562	296 / 56,520

V. SCIENTIFIC OVERVIEW

DIAGNOSTIC IMAGING RESEARCH BRANCH

The Diagnostic Imaging Research Branch has grown rapidly since its inception in 1982. The number of grants awarded has increased from 31 to the present figure of 98. Correspondingly, the number of grant applications has increased from 50 to 218 emphasizing the increasing visibility of this Branch. At this time there are five contracts designed for "Comparative Clinical Magnetic Resonance Imaging Studies."

Several major areas in which the Diagnostic Imaging Branch is supporting research stand out.

Sensitivity, Specificity of Diagnosis

Perhaps the most important effort in the Diagnostic Imaging Research Branch is and will be to improve sensitivity and specificity of tissue diagnosis. This effort represents a consensus arrived at by the "Directions in Research" workshop described above. It is, further, reflected in a Program Announcement to promote study of correlation of magnetic resonance imaging and spectroscopy, issued during FY85. The Program Announcement is entitled, "Combined In Vivo Application of Multi-Nuclear Magnetic Resonance Imaging and Spectroscopy."

Three grants are supporting efforts to specifically diagnose tumors using radio-labeled substances. One employs monoclonal antibodies. Another employs estrogen receptors in breast tumors using radioactive isotopes. A third project has as its goal the development of Technetium 99m complexes which localize in estrogen dependent tumors as a result of high affinity binding to estrogen receptors.

Magnetic Resonance Imaging

Magnetic Resonance Imaging is a major breakthrough in the imaging field. It is providing anatomical information not previously available with other modalities. The Branch has an ongoing three-year contract with five Institutions which is directed toward comparing MRI with other current state-of-the-art modalities. The protocols are designed to evaluate musculoskeletal tumors, brain neoplasms, uterine neoplasms, lung cancer, metastatic liver disease and cervical myelopathy.

Supported by grants are other studies employing magnetic resonance. Of special interest is a study of metabolic processes by imaging of nuclei other than protons and the correlation of the images with spectroscopic data, e.g., identification of a phosphorus metabolite and localization of the metabolite anatomically. Such studies will permit assessment of metabolic and physiologic functions, and are expected to allow determination of response of cancer to therapy. Of major importance is the further pursuit of specificity of diagnosis by spectroscopic technology. Specific identification of carcinomas of lung and breast has already been achieved. In still another direction, - employing a paramagnetic contrast agent, - gadolinium-labeled monoclonal antibodies have provided enhanced contrast of melanoma metastases.

Grants have been awarded to evaluate T_1 and T_2 relaxation times. These studies represent an attempt to differentiate malignant tissue from benign by this parameter of magnetic resonance imaging.

Breast Disease

The work in breast disease in this Branch is aimed at the development and evaluation of new imaging systems for early detection and characterization of benign and malignant disease. Presently there are 10 grants based on ultrasound (ultrasound computed tomography, improvements in image quality, test phantoms, and propagation properties of sound in breast tissue); three studies on magnetic resonance - tissue properties and test phantoms; three on visible and infrared transmission imaging of the breast (diaphanography); one on low dosage mammography and two on radionuclide approaches employing estrogen receptor techniques.

Ultrasound

Other ultrasound studies are directed at noninvasive detection and differentiation of liver diseases using more advanced tissue characterization methods. Several investigators continue to conduct bioeffects research in an effort to identify and reproduce any nonthermal effect at diagnostic levels of exposure which might be construed to have a potentially adverse effect on biological organisms. Thus far, no definite adverse effects have been found.

Instrumentation

Digitized Radiography

The problems involved with achieving high quality radiographs for cancer diagnosis is nowhere more evident than in the conventional chest radio-

graphy. Several grants funded by DIRB to improve chest imaging use various approaches. One of the grants employs "Scanning Equalization Radiography." This technique exposes the patient with a non-uniform field of radiation to compensate for variations in tissue thickness over the chest. Another grant compensates for variations in tissue thickness by fabricating a patient-specific x-ray beam filter. The filter directs a spatially variable amount of radiation to each part of the chest, resulting in an optimal image contrast for all regions of the chest. Still a third grant implements a film scanner-digitizer for computerized image processing of conventional chest radiographs. These efforts to enhance and improve images of the conventional variety are most important since they affect the oldest and yet the most used radiologic examination in current radiology departments.

Image Perception

Image perception is receiving attention. The recent workshop referred to above indicated the need to evaluate the newer technologies that are evolving which provide largely video images as opposed to film images. This effort, currently supported by five grants, will play an important role in evaluating the usefulness of the newer technologies.

PET and SPECT

Eighteen grants support the engineering and physics aspects of Positron Emission Tomography (PET) and Single Proton Emission Computed Tomography (SPECT). PET is directed primarily toward function as opposed to anatomy, although anatomy is well shown in the image. However, there is much room for improvement of images and efforts are being made toward building PET systems with high resolution and high sensitivity.

Single Photon Emission Computed Tomography using a different physical principle would provide essentially the same type of information that Positron Emission Tomography furnishes, namely, function in an anatomical setting, but at greatly reduced cost. Research in PET and SPECT focuses on the use of radiolabeled metabolic compounds for the study of physiologic and biochemical parameters in vivo. There are grants to support study of the quantitative capability of SPECT. A major factor hindering development of SPECT is the unavailability of suitable gamma emitting isotopes which SPECT requires (as opposed to the positron emitting and much more expensive and shorter lived isotopes used with the PET modality). An effort to further this important research has resulted in concept approval for an RFP to develop radiopharmaceuticals for SPECT.

SUMMARY

Overall, the Diagnostic Imaging Branch budget of FY85 supports grant-in-aid mechanisms at a level of about $16,000,000 and contracts at $1,000,000. There are five program project grants (P01), almost 100 regular grants (R01), two new investigator awards (R23), and one conference grant (R13). Our Small Business Innovative Research (SBIR) program (25 grants) is the largest at NIH. Program project grants constitute approximately 15% of our total grant budget. The funded grants have almost tripled in number in the last three years and the total budget in FY85 is more than three times that of FY82.

RADIOTHERAPY DEVELOPMENT BRANCH

The Radiotherapy Development Branch continues to administer a large program of basic and clinical research related to cancer treatment. The scientific disciplines represented are radiation oncology, radiobiology, radiation chemistry and radiation physics.

Major areas of research in terms of funding are particle radiotherapy, hyperthermia and associated biology, radiosensitizers, intraoperative radiotherapy, photodynamic therapy and radiation physics.

Particle Radiotherapy

Support of particle radiotherapy constitutes the largest dedicated portion of the RRP budget. Included are investigations at six neutron facilities, one proton, and one helium/heavier ion facility. Neutron researchers are completing Phase I/II dose searching protocols. Encouraging preliminary results with neutron therapy are suggested for prostate and salivary gland tumors. Research with heavier ions, helium and protons continues. These particles provide a superior dose localization characteristic which enables sparing of tissues away from the tumor and concentration of dose within the tumor. Heavy ions also provide the radiobiological advantage of greater cytotoxicity for hypoxic tissue.

Hyperthermia and Radiotherapy

Much activity continues in the area of regional hyperthermia with radiotherapy. Efforts range from basic to clinical. Significant support has been given the two common modalities for producing hyperthermia (electromagnetic radiation (radiofrequency and microwave) and ultrasound). The major problem that remains is the heating of deep seated tumors. Phase I and II clinical research (treating superficial tumors) suggests that this modality could have a significant effect with a variety of tumors.

Intraoperative Radiotherapy

Three contractors have developed guidelines for the treatment of intra-abdominal malignancies in patients during Phase I studies. Techniques have varied. Generally pelvic tumors have been treated with intraoperative therapy and the removal of bulk tumor at surgery, together with either pre- or post-operative external radiation. Rectal tumors treated in this fashion have shown early results (13% failure of 3-year local tumor control) considerably improved over the presently conventional surgery plus external radiation (44% local control failures).

Other organs/areas that have been treated are pancreatic carcinoma, retroperitoneal sarcomas, para-aortic nodes and gastric carcinoma and regional nodes. There are good indications that IORT may offer substantial improvement in local control and palliation in advanced disease situations, and that clinical trials are now in order.

Radiosensitizers

Basic research with several radiosensitizers has continued. The screening program has produced leads in two non-nitro (and non-neuropathic in contrast to the imidizoles) classes of compounds, - the quinoxaline line and the benzamide line. SR-2508, a nitro-imidazole, in Phase I clinical trials has been found to be 2.5 to 3 times less toxic (peripheral neuropathy) than misonidazole, (the gold standard of the radiosensitizers). Phase II and III studies are being planned.

Phase II clinical trials to evaluate misonidazole as a chemosensitizer in combination with 1) melphalan and 2) cis-platin are underway. Investigation of the mechanisms of chemopotentiation by radiosensitizers when used in combination with conventional chemotherapeutic drugs has shown that the magnitude of the chemopotentiation is oxygen sensitivity-related, being maximal under anoxic conditions.

Photoradiotherapy

Basic research in this field is active. A di-hematoporphyrin ester with an affinity for plasma lipoproteins has been identified as an active tumor localizing component of hematoporphyrin. This will permit pursuit of more effective analogues which have less skin photosensitization effect than currently used hematoporphyrins. Skin photosensitization has been the major toxicity associated with PDT.

The biochemical mechanisms involved in tumor cell cytotoxicity are also being investigated. Mitochondrial function can be severely injured by PDT in a dose and time related relationship. It would appear that the hydrophobic environment of the mitochondrial membrane is conducive to localization of hematoporphyrin and that impaired mitochondrial function may offer the basis for the cytotoxicity that occurs.

Hyperthermia has been found to be strongly synergistic with PDT, especially when given immediately post-PDT.

Phase I and II clinical trials have shown good local control and palliation in certain endobronchial, head, neck, bladder and eye tumors. Several centers feel that limited site-specific and tumor-specific Phase III randomized clinical trials are ready to begin.

Radiation Physics

Continuing support is provided for characterization of external photon and electron beams. Data indicates that there are different microdosimetric properties in the deposition of the energy of these beams in tissue.

Microdosimetry of intracellularlly deposited radionuclides shows that radionuclides that decay by electron-capture with the subsequent production of many low energy electrons concentrate their damage in small sub-cellular volumes. Current schemes of dosimetry neglect this local concentration. This is an area that is complex and not well understood at the present time and which requires much further study.

Graphite and tissue equivalent liquid calorimeters are being developed to study the deposition of energy in tissue by neutrons. Work is underway to develop a neutron dosimetry standard for the neutron clinical trials.

VI. RADIATION RESEARCH PLANS FOR 1986

The Diagnostic Imaging Research Branch looks forward to a stimulating research future in two complimentary areas -- 1) the improvement of sensitivity and specificity of diagnosis by imaging and closely related methodology (e.g.,- magnetic resonance imaging and spectroscopy, radiolabeled monoclonal antibody techniques, radiolabeled organ receptor techniques) and 2) the inception of multi-institutional imaging trials for Phase III studies. The latter cooperative group method of investigation permitting the participation of multiple institutions in specific protocols will enable a reasonably rapid accumulation of data and the clinical evaluation of Phase I and II trials as they apply to the improvement of sensitivity and specificity of diagnosis by imaging.

The advent of computed tomography and magnetic resonance is expected to revolutionize the field of diagnostic radiology. Many previously useful radiologic procedures will become obsolete as diagnosis becomes simplified. Digitization of conventional radiologic studies will greatly improve diagnosis. Sharp advances in ultrasound with improved instrumentation (by industry) and the practicality to determine functional abnormalities made possible with new radiopharmaceuticals employing PET and SPECT methodology are expected to become a reality. The ultimate objective is specific tissue diagnosis obtained noninvasively.

The multi institutional imaging trials of the future should deal with additional problem areas such as algorithms for the development of diagnostic procedures and cost efficacy.

The Radiotherapy Development Branch looks forward to increased therapeutic efficacy in the following areas:

- With the achievement of effective devices for heating deep-seated tumors, more effective treatment of resistant tumors can be expected. Extensive radiobiologic studies in hyperthermia are in progress and will assist greatly in utilizing this methodology.

- Radiosensitization of tumor tissue can be expected to improve efficacy of radiotherapy as the availability of additional or more effective radio sensitizers becomes a reality. The effort to combine chemopotentiation with radiosensitization is intriguing and will be pursued enthusiastically.

- Neutron, Proton and Photodynamic Therapy promise to be exciting additions to the radiotherapy armamentarium. These have limited application but results are effective with certain tumors.

The Diagnostic Imaging Research Branch (DIRB), Radiation Research Program (RRP), DCT, NCI, supports research leading to the development of radiologic instrumentation and methodology to improve diagnosis of cancer and other diseases. This includes the rapidly developing nonionizing modalities such as magnetic resonance imaging (MRI), diaphanography and ultrasound, as well as improvements in x-ray computed tomography (CT), positron emission tomography (PET), single photon emission computed tomography (SPECT) and other research in nuclear medicine and nuclide imaging.

The Diagnostic Imaging Research Branch is concerned with research leading to new and relevant information in the field of medical imaging. Current research is directed toward several major areas. The development of new and improvement of existing imaging devices utilizing both ionizing and nonionizing radiation technologies to enhance diagnostic imaging for cancer and other diseases and at the same time minimize the radiation exposure of patients has high priority. A second area is support for production of a variety of contrast agents for imaging including new nontoxic low-cost contrast media, synthesization of new radiopharmaceuticals, production of paramagnetic agents for magnetic resonance imaging and contrast agents for use with ultra sonography. A third area is the development of methodologies for the use of important radiolabeled macromolecules in biological, physiological, and clinical research especially as related to imaging of both normal and malignant tissues. Perhaps the most significant area of support is the evaluation of the clinical application of new scientific advances for diagnosis and monitoring of therapy. Furthermore, the diagnostic imaging program supports image perception research and research into the bioeffects of various energies for diagnostic imaging, including ionizing radiation, ultrasound, radiofrequency waves and high magnetic fields.

Within the several fields described, a major goal of the Diagnostic Imaging Research Branch is image enhancement for cancer diagnosis. Improvement of sensitivity and specificity of tissue diagnosis is a related objective of great interest. Research is being encouraged in the field of tissue characterization for various imaging modalities, especially MRI and Ultrasound. These efforts are evident in the many workshops sponsored by DIRB as well as Program Announcements soliciting applications and proposals.

Studies of metabolic pathways in both normal and tumorous tissues using radiolabeled compounds constitute a significant portion of our nuclear medicine program. Emphasis is being placed on the use of monoclonal antibodies (MCA) for diagnostic purposes. The efficacy of monoclonal antibody fragments as carriers of radiolabeled isotopes for diagnostic imaging or for radiotherapy is a significant tool to combat cancer and other diseases. Research supported by this Branch showed that administration of ^{131}I labeled antibody intravenously administered, resulted in imaging of metastatic choriocarcinoma to lung and abdomen in a woman with recurrence, heralded only by rising serum human chorionic gonadotropin (HcG) levels. After the metastases were found with the radiolabeled antibody, they became clinically evident to CT scanning. This result

holds promise for detecting and treating recurrent metastatic choriocarcinoma at the earliest possible stage, with similar implications for other neoplasms on the horizon.

Encouraging results of Technetium-99m labeling of monoclonal antibodies have been obtained by one of our grantees. He was able to develop a scale up synthesis of a diamide dimercapto (N_2S_2) ligand that has a side chain containing an activated ester for conjugation to proteins. These results indicate good yields of Tc-99m labeled antibody with retention of immunoreactivity. Another project has as its goal the development of Tc-99m complexes which localize in estrogen dependent tumors as a result of high affinity binding to estrogen receptors. Further research is certainly needed in this promising area.

The past few years have seen a revolutionary growth in the development and application of magnetic resonance imaging (MRI) systems for clinical diagnostic use. Over 100 systems are now operating in the United States, and the list is rapidly growing throughout the world. This represents a very large capital investment in new diagnostic equipment, but it also offers a new modality for noninvasive detection and diagnosis of disease without ionizing radiation, the ability to image any cross-sectional plane within the body, great flexibility to alter image contrast electronically at will, and new capabilities for imaging and characterizing soft tissues throughout the body.

In recognition of the need for carefully planned research into the capabilities and limitations of this new modality, the Diagnostic Imaging Research Branch is carrying out a program of studies to compare MRI with other current state-of-the-art modalities. Three-year contracts were awarded to each of five institutions for a total of $3,400,000. These contractors have collaborated as a Working Group to develop criteria and guidelines for use of clinical MR imaging, to formulate plans and protocols for comparative studies, and to perform and evaluate comparative diagnostic studies for detection and diagnosis of musculoskeletal tumors, brain neoplasms, cervical myelopathies, uterine neoplasms, lung cancer, and liver metastases. Other protocols are also under consideration.

Anatomical magnetic resonance images currently being produced on the basis of protons (or hydrogen nuclei) within the tissues are of excellent diagnostic quality and provide new ways of visualizing nearly all parts of the body. High magnetic fields permit imaging of nuclei other than hydrogen, e.g., ^{31}P, ^{123}Na, ^{19}Fl, thus opening up further possibilities for new information not previously obtainable. Further, these nuclei can be identified spectroscopically. Magnetic resonance spectroscopy will enable both research and clinical personnel to study physiologic functions (e.g., metabolism and blood flow) and may allow assessment of the responsiveness of tumors to chemotherapy, radiation therapy, hyperthermia, and other interactions. A Program Announcement entitled, "Combined In Vivo Application of Multinuclear Magnetic Resonance Imaging and Spectroscopy" is being made this year to invite applicants to participate in this vital area of magnetic resonance research.

One of the most interesting new MRI developments is a technique which permits the computation of a magnetic resonance image of optimum contrast (to visualize a certain body part) simply by computer optimization of the data taken

from a couple of initial MR views without requiring repeated trial and error "exposures." The "computed image" can, moreover, be calculated and displayed without having to re-image the patient, thus minimizing patient examination time and inconvenience, improving patient through-put, and thereby reducing examination costs.

For several years it has seemed apparent that the new MR imaging modality might also permit noninvasive differentiation between normal and malignant tissue areas by the concurrent measurement of the so-called magnetic relaxation times T1 and T2 in the areas of the image. This has not yet been found possible in exact terms because of overlapping values between normal and malignant tissues, heterogeneity of samples, and other unknown factors. In order to provide scientific understanding of the factors that determine T1 and T2 values in biological tissues, several basic research studies have now been undertaken.

One-third of all the non-ionizing research programs in DIRB relate to detection and diagnosis of breast disease. This strong involvement points up the fact that, despite demonstration of more satisfactory results for clinical therapies in recent years, breast disease diagnosis remains a serious challenge. Although the new magnetic resonance imaging systems bring new capabilities to breast cancer diagnosis, these instruments are so expensive, and so few, that they are not used at this time for screening on a national basis. For early detection, we must depend on the recommended combination of (1) periodic self and/or physician's physical exam and (2) x-ray mammography, coupled (where available) with other adjunctive modalities such as ultrasound and diaphanography (method for scanning the breast with light of visible and near-infrared wavelenghts).

Presently there are ten grants based on ultrasound (ultrasound computed tomography, improvements in image quality, test phantoms, and study of propagation properties of breast tissue); three on magnetic resonance (tissue properties and test phantom development); three on diaphanography; one aimed at low dose x-ray mammography; and two on radionuclide approaches to breast disease assessment.

One new experimental ultrasound imaging system developed under a DIRB Small Business Innovative Research grant has recently demonstrated in a test phantom that it may soon be possible to detect and image microcalcifications of the order of 0.5 mm or less, making ultrasound an even more valuable adjunct in breast imaging. In another program, three institutions are beginning a comparative clinical evaluation of diaphanography of the breast.

Other ultrasound studies are directed at noninvasive detection and differentiation of liver diseases using more advanced tissue characterization methods. Several investigators continue to conduct bioeffects research in an effort to identify and reproduce any non-thermal effect at diagnostic levels of exposure which might be construed to have a potentially adverse effect on biological organisms, thus far with no success.

The problems of achieving high quality radiographs for cancer diagnosis is nowhere more evident than in the chest. In the area of digitized radiography, several grants have been funded by DIRB to improve chest imaging using various

approaches. One of the grants uses Scanning Equalization Radiography (SER) as a new method of improving chest imaging. This technique exposes the patient with a non-uniform field of radiation to compensate for variations in tissue thickness over the chest and variously dense tissues (organs within the chest). Another grant employs a computerized heavy metal printer technique which, based on a low-dose electronic test image, fabricates a patient-specific x-ray beam filter. When placed in the x-ray beam for final film exposure, the filter directs a spatially variable amount of radiation to each part of the chest. This results in optimal image contrast in all regions as determined by experiments involving test objects. A third grant implements a film scanner digitizer for computerized image processing of conventional chest radiographs. Various algorithms to enhance imaging contrast on films of cancer patients at risk for lung metastases are being developed. These are but a few examples of research to improve imaging of the oldest, yet the most used method in radiology departments.

Research in image perception seeks to increase the diagnostic accuracy of image-based diagnoses by evaluating all the factors which influence diagnostic decision in order to reduce human variability in the image-reading process. This Branch is currently supporting five such grants. One project has developed a theoretical model to provide a basis for using the radiologists' confidence-rating judgments to determine the relative magnitudes of several components of variability in image-based diagnosis. The results of using this model on computer-simulated images by expert mammographers to distinguish malignant from benign breast lesions show improvement in their diagnostic accuracy. Another project has investigated the human ability to detect lesions on CT images. They have simulated hepatic lesions (spheres) and blood vessels (cylinders) and superimposed such lesions on single and multiple images obtained from actual CT scans of water phantoms and of patients with normal livers. They were able to predict observers' detectability by simple manipulations of size, attenuation, image noise ratio and display. In a third project observers of chest images were presented with x-ray images containing nodules visible to the central vision but not to the periperhal vision. This resulted in 20 percent improvement in nodule detection over natural viewing.

Engineers and physicists constitute a good number of our grantees. They are supported to improve various modalities. Grants in the area of Positron Emission Tomography (PET) cover areas such as development of a sensitive detector using multiple gadolinium orthosilicate (GSO) scintillation crystals. Another supports building a PET system with high resolution and high sensitivity. This newly designed PET, in the researchers words, will be able to compete with CT as well as MRI for picture quality.

Single Photon Emission Computed Tomography (SPECT) is another engineering area of great interest to our Program. The significance of SPECT is that it can give tomographic images using simple gamma-emitting radioisotopes rather than requiring short-lived positron emitters as does PET scanning. The use of PET requires immediate physical availability of a cyclotron to produce the short-lived isotopes and the instrumentation is dedicated to this purpose only.

The SPECT scanner, on the other hand, can be used for routine nuclide studies in addition to tomography. However, 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 radio-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 normal and malignant tissues for the purpose of cancer detection.

Two grants are funded in this area to solve one of the most important issues in nuclear medicine, that is, the quantitative capability of (SPECT). For example, measurements of important physiological parameters such as regional brain blood flow requires that the radiopharmaceutical activity within the organ be accurately quantified. One of the major factors reducing the accuracy of SPECT quantification is the unavoidable detection of gamma rays scattered within the patient. Researchers supported by this program are succeeding in compensating for image inaccuracies resulting from scattered photons by mathematical and subtraction approaches.

In order to encourage research in this vital area, a workshop entitled, "Functional Imaging with SPECT" was sponsored by DIRB and held last year. The prime recommendation of this workshop was to support development of radiopharmaceuticals using Tc-99m and I-123 which could be incorporated into biological systems as probes of physiologic and biologic processes. This has resulted in an RFP entitled, "Development and Improvement of Radiopharmaceuticals for Employment with Single Photon Emission Computed Tomography."

Overall, the Diagnostic Imaging Program budget for FY85 is approximately $16 million supporting research using grant-in-aid mechanisms and over one million to support five contracts in the area of MRI research. There are 5 program project grants (P01), and about 100 regular grants (R01), two new investigator awards (R23), and one conference grant (R13). Our Small Business Innovative Research (SBIR) program (25 grants) is the largest at NIH of this newly developed research program. Program project grants constitute approximately 15% of the total grant budget.

The funded grants have almost tripled in number in the last three years and the total budget in FY85 is more than three times that of FY82.

FY85 Annual Report Summary
Radiotherapy Development Branch

The Radiotherapy Development Branch (RDB) 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.

Areas of basic research supported by RDB have generated leads for promising new treatment modalities that are currently being tested in clinical trials. Among these are particle therapy, intraoperative radiotherapy, radiation modifiers, and photodynamic therapy. Radiation modifiers include 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.

Major areas of research in terms of funding are particle radiotherapy, hyperthermia, and general radiobiology. Substantial support is also provided for the development of radiosensitizers and radioprotectors, intraoperative radiotherapy, photodynamic therapy, and radiation physics.

The largest dedicated portion of the Radiotherapy Development Branch budget supports particle radiotherapy from very basic (nucleus - nucleus interaction cross section) to fully clinical research. This program goes back a decade and now funds eight clinical research facilities; six neutrons, one proton, and one helium/heavier ion. With the completion of FY 1985, all neutron programs are now contract funded. The group of neutron clinical researchers are completing a series of Phase I/II dose searching protocols and in FY 1986 will commence a series of about six Phase III protocols which are expected to be completed before 1990. Encouraging preliminary results have emerged from previous studies especially for prostatic and salivary gland tumors. Clinical research on heavier ions continues at Lawrence Berkeley Lab with selected Phase III expected to commence in FY 1986 in addition to the continuation of certain Phase I/II trials. Research on helium ions and protons continues, respectively, at Lawrence Berkeley Lab and the Harvard Cyclotron Lab. Exploitation of the superior dose localization properties of these particles continues to be investigated for appropriate tumors, uveal melanomas being the classic example. It is expected that in FY 1986 discussions will begin between NCI staff and several potential applicants for hospital-based proton therapy facilities.

The most rapidly growing area of research supported by the Radiotherapy Development Branch continues to be loco-regional hyperthermia in conjunction with radiotherapy. A broad program of basic research has been established

to complement a vigorous clinical research program. One researcher reports that heat may enhance antigenicity of cells which would provide a molecular explanation for the frequently stated impression that hyperthermia stimulates immune response. Another researcher reports that membrane lipids appear to be key determinants of thermosensitivity. A number of investigators are examining the role of heat shock proteins. There are indications that they may play a pivotal role in protecting cells from thermal damage. Other researchers are investigating the potential of various chemicals to sensitize or protect cells from heat damage. Favorably manipulating tumor and normal tissue blood flow to enhance tumor heating is also being vigorously researched. Because electromagnetic (radiofrequency and microwave) and ultrasonic radiation are the most common modalities for external hyperthermia, considerable research is ongoing into their interaction with normal and tumor tissues. The major obstacle to exploiting the encouraging clinical information on hyperthermia is the very difficult problem of heating the more common deep-seated tumors. In simplest terms, the poor penetrating and focusing properties of electromagnetic and ultrasonic radiation make deep tissue heating difficult. The problem is further complicated by increases in blood flow to heated areas in response to heating. The many facets of this overall problem are currently being investigated by NCI-supported researchers. Despite all the difficult problems yet to be solved, Phase I and II clinical research strongly suggests that appropriate combinations of heat and radiation could have substantial impact on a variety of tumors.

Contract-supported efforts for the in vitro and in vivo screening of a large number of compounds as potential radiosensitizers has resulted in several new and interesting leads. Quinoxylines and benzamides have shown significant activity to warrant patenting these compounds as radiosensitizers. These clinical classes are being developed further by the contract-supported synthesis contractor who is making and testing analogues in search for the optimal compound of each class. Several other classes of compounds have also shown in vitro activity and will be tested in vivo when sufficient material becomes available.

Basic research in the field of radiosensitizers and radioprotectors is providing a better understanding of the mechanisms and structure relationships that play a role in radiobiological activity. It has been shown that the reduction of intracellular glutathione with buthionine sulfoximine (BSO) increases the radiosensitizing effectiveness of Misonidazole and SR-2508. This finding has potential widespread clinical application. It has been shown also that hypoxic tumor cells are radioresistant at low doses of radiation per fraction (1-4 Gy) but that this radioresistance can be overcome with the hypoxic cell radiosensitizer SR-2508 in multifraction experiments in vivo. The role of hypoxia in the response of tumors to radiation and drugs was demonstrated by the finding that tumors of mice made acutely anemic became radioresistant, but returned to normal radiosensitivity within 1-2 days. Retransfusion to normal, or administration of perfluorocarbon emulsion with 100% oxygen breathing increased the radiosensitivity of the tumors well above that in normal mice. The repair of potentially lethal damage caused by alkylating agents was found to be inhibited in vitro by the so called PLD repair inhibitors which, at least for alkylating agents, alter pharmacokinetics so that the inhibitors considerably enhance alkylating agent toxicity in normal tissues as well as tumors. Investigation of the mechanisms of chemopotentiation

by radiosensitizers when used in combination with conventional chemotherapeutic drugs has shown that the magnitude of chemopotentiation is oxygen sensitive, being maximal under anoxic conditions. Efforts are underway to synthesize new and more effective chemopotentiating compounds.

The Phase I clinical trial of the radiosensitizer SR-2508 has been completed. Approximately 2.5 to 3 times more SR-2508 can be administered than Misonidazole before the dose-limiting peripheral neuropathy is seen. Pharmokinetic studies indicate that sufficient tumor tissue levels of the drug are achieved to radiosensitize. Phase II-III studies are being planned in selected anatomic sites for SR-2508. Phase II clinical trials are also in progress to determine the effectiveness of the radiosensitizer misonidazole as a chemosensitizer. Misonidazole is being used in combination with melphalan or cis-platinum.

Less effort has been put into the area of radioprotective agents. Basic studies, both in vitro and in vivo, are attempting to define the basic mechanisms involved. The radioprotectors still being utilized are those aminoalkylthiols which were developed at the Walter Reed Army Institute of Research. One of these radioprotectors, WR-2721, is completing a Phase II clinical trial and will probably be moved into a randomized Phase II-III trial in the near future.

Three contractors constitute a working group established to develop guidelines for the treatment of intra-abdominal malignancies with intraoperative radiotherapy (IORT). The potential advantage of this technique is that many radiosensitive normal structures can be removed from the radiation field during a surgical procedure so that a single high radiation dose can be delivered directly to the tumor or tumor bed during surgery 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 improved control of many localized, yet difficult to treat, malignancies. The results of the Phase I clinical trials conducted thus far indicate that about 25% of the patients treated experience severe or life-threatening complications. Most of these complications, however, are manageable and improve over time. There are some indications that IORT may offer substantial gains in local control and palliation when compared with other treatment options in cases of similarly advanced disease. Support of Phase II-III clinical trials using IORT as adjuvant therapy is expected for studies being planned by members of the Radiation Therapy Oncology Group - a multi-institutional clinical trial group.

Pre-clinical studies in intraoperative radiotherapy have been directed to the long term responses of canine paraaortic tissues. The most important finding has been the sensitivity of peripheral nerves to large single doses of irradiation. Many of the structures irradiated, such as ureter, bladder, kidney and peripheral nerves may tolerate doses of no greater of 20 Gy given as a single dose. The great vessels, aorta and vena cava, appear to be more tolerant. These observations resulted from histologic studies done at six months following irradiation.

Preliminary indications are that damage to those structures may be significantly greater after two or three years. Studies are ongoing to evaluate much later effects, but the response of the peripheral nerves and the changes seen

in the wall of the aorta provide information which must be viewed as cautionary in the use of intraoperative radiotherapy.

Basic research into better physical characterization of various ionizing radiation modalities is continuing. Support is being provided in external photon and electron beams. The spectroscopic and microscopic data confirm that electron and photon beams have distinctly different microdosimetric properties in the deposition of their energy in tissue. The testing of a polystyrene "non-isolated sensor" (NIS) calorimeter has demonstrated a high degree of precision of the absolute absorbed dose measurement and will permit the evaluation of the accuracy of ion chamber measurements in electron beams. A study on the therapeutic and toxic action of intracellularly deposited radionuclides has shown the importance of microdosimetry. Radionuclides that decay by electron capture with the subsequent production of many low energy electrons concentrate their damage in small, subcellular volumes. Current schemes of dosimetry neglect this local concentration. The methodology of dose calculations is being developed for cancer therapy from internally deposited radioactively labelled antibodies directed to tumor associated and/or tumor specific antigens which could have wide applicability in the experimental treatment of many cancers.

The deposition of energy in tissue by neutrons is being investigated, as well, through the development and study of graphite and tissue-equivalent liquid calorimeters and the study of neutron scattering by cellular elements to develop models of energy deposition. Work is well underway to develop a neutron dosimetry standard for the neutron clinical trials.

Evaluation is being performed on gynecologic intracavitary applicator attenuation in order to improve dosimetry calculations by treatment planning computers.

Treatment planning with computers is being investigated in several areas. One grantee is developing multi-dimensional treatment planning capability for conventional sources of radiation. Calculational and display techniques have been developed for 10 and 25 MeV photon beams. Dose uncertainty calculations that account for the effects of patient motion and calibration error in CT data have been developed and applied. Techniques for comparison of rival treatment plans and algorithms for comparison of various diagnostic imaging modalities are under development. Multi-institutional contracts in computerized treatment planning are evaluating and seeking to improve techniques with particle beams (neutrons, protons, helium ions and heavy ions) and developing criteria, guidelines and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. Future contracts are planned for electron beam and interstitial source treatment planning. These contracts should lead to a comprehensive evaluation of computerized treatment planning and point the way to future advances in this area.

Support continues for the construction of new neutron therapy facilities and continued support of present facilities. Within a few months as many as six facilities will be conducting Phase III clinical trials of neutron therapy versus photon therapy for selected cancers. Within a few years the efficacy of neutron therapy should be elucidated.

The Radiological Physics Center continues to provide physics support for dosimetry quality assurance for NCI funded inter-institutional clinical trials. Efforts center primarily on dosimetry review visits to participating institutions, periodic calibration checks by mailed thermoluminescent dosimeters (TLD), and review of radiation therapy treatment records for individual patients entered onto cooperative clinical trials.

The end result of much of the foregoing effort is treatment delivery to the patient. Attention to this crucial link in the cure of the cancer patient is also receiving attention. A grantee is constructing and testing a scanning diode array imager to obtain visual confirmation that the area of the patient being treated is the same as the area prescribed by the physician.

The Board continues to support a major portion of Radiation Biology research in the U.S. It is all directly or indirectly related to improving radiation therapy as a treatment modality. Tumor and normal tissue radiation biology at the molecular, cellular, tissue and whole animal levels continue to be areas of vigorous investigation. More specific topics include: time-dose fractionation, hyperfractionation, long-term consequences of post-mastectomy irradiation, inducible cellular repair, radiation and the immune response to cancer, cellular heterogeneity, prognostic indicators, repair kinetics, and low dose rate effects. While many of these topics seem far removed from radiation therapy, one can readily be convinced otherwise by investigating the genesis of clinical research topics of current interest. Particle therapy, hyperthermia, radiation sensitizers, photodynamic therapy, etc., all started from relatively basic research observations.

The RDB still relies heavily for guidance on new research initiatives on the Interdisciplinary Program for Radiation Oncology Research, developed with NCI support in 1984 by a group of leading scientists under the aegis of the American College of Radiology Commission on Cancer.

During FY 1985, the RDB sponsored four workshops on topics of potential future solicited research: 1) a Planning Workshop for Future Patterns of Care Studies, 2) Phase III Clinical Trials Using Photodynamic Therapy - Associated Problems, 3) Normal Tissue Tolerance to Combined Modality Therapy Using Radiation and Chemotherapy, and 4) Verification of Patient Treatment Fields in Radiation Therapy.

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